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PHYTOPATHOLOGY

VOLUME X NUMBER 3

MARCH, 1920

BACTERIAL BLIGHT OF SOYBEAN

FREDERICK A. WOLF

WITH FIVE FIGURES IN THE TEXT

INTRODUCTION

For several years, especial attention has been given at the North Carolina Agricultural Experiment Station to the diseases of soybean, *Soja max* (L.) Piper. The most important diseases of this crop within the State have been found to be Fusarium blight or wilt, caused by *Fusarium tracheiphilum* E. F. S., and bacterial blight. Careful investigation of the former of these diseases has been made by Cromwell (2), whereas all existing published accounts of the latter have been found to consist of fragmentary notes.

The present studies on bacterial blight have been directed mainly toward the isolation of the causal organism, its identification, and the procurement of evidence of its pathogenicity. In addition, certain field observations and laboratory studies have been made during the past three seasons which further contribute to our knowledge of this disease and are consequently recorded in the present report.

HISTORICAL ACCOUNT

The first mention of the occurrence of bacterial blight of soybeans appears to have been made by Heald (3, 4) in 1905. His account consists of a brief description of the appearance of the disease, with the suggestion that it is of bacterial origin. Subsequently, Smith (7) and Clinton (1) report the occurrence of a bacterial leafspot of soybean, and the latter author states that the disease in Connecticut is like the one described by Heald (3, 4) in Nebraska. Apparently, the only critical investigation of bacterial blight which has been made was conducted in Wisconsin by Johnson and Coerper (5). They isolated a white bacterial organism refer-

able to the genus Pseudomonas, which they proved to be pathogenic. However, the detailed report of these isolation and inoculation studies and description of this organism appears never to have been made.[1]

A brief report of the present author's work in manuscript form, in which the causal organism is designated *Bacterium sojae* n. sp., was prepared by Tisdale (8). The manuscript on bacterial blight, dealing with the investigations conducted during 1917 and 1918, has been supplemented and modified by studies made during the past season and is presented herewith.

APPEARANCE OF THE DISEASE

Bacterial blight of the soybean has been observed upon plants varying in age from seedlings to mature plants. It may appear upon the cotyledons or upon the true leaves in any stage of their development. Apparently stems and fruits are free from invasion by the parasite. The disease is, furthermore, widely distributed throughout the State, since it has been noted in practically every soybean field which has come under observation.

The first evidence of the disease which has been noticed is the presence of lesions on the cotyledons just as they are emerging from the ground. The lesions present at this time are usually marginal and are in the beginning little different in color from the normal tissues. They rather rapidly become brown, however, and meanwhile the tissues have shrunken and collapsed and the area of invasion may have enlarged, extending backward toward the hypocotyl. In extreme cases both cotyledons are de-

[1] Since this manuscript was submitted for publication, a report of investigations on "Bacterial Blight of Soybean" (Coerper, Florence, M. Jour. Agr. Res. v. 18, No. 4, p. 179–194, pls. 12–18, one colored plate and one text figure, 1919), in Wisconsin, has appeared. This disease apparently differs in several respects from the one occurring in North Carolina in which, (1) the diseased areas are *typically* not so dark and usually remain intact; (2) the dried exudate is grayish and not brownish; (3) leaf parenchyma and cotyledons only are involved, since careful search during three seasons has failed to reveal lesions on petioles, stems, legumes, or seeds. The seed are believed to become contaminated by the washing of the parasite from the leaves only after the dehiscence of the legumes, and apparently normal seed, when planted, give rise to seedlings whose cotyledons are affected; (4) colonies of *Bacterium sojae* are smaller than *Bacterium glycineum* and the former organism is furthermore smaller than the latter. No strains of *B. sojae* with umbonate center, with wrinkled or convoluted surface, or which produce brown pigment, grow in the closed arm of fermentation tubes, or produce acid from the several sugars, have been isolated. At least a dozen strains have been isolated by the writer and three of them have been used, with concordant results, in the cultural studies.

The name Southern bacterial blight of soybean is therefore tentatively applied to the disease under consideration.

stroyed and the hypocotyl is involved, thus causing the death of the seedling. Species of bacteria other than the parasite are always present in mature lesions on the cotyledons. In some instances one cotyledon only is involved in decay. Lesions which are marginal in origin are commonly irregular in outline, whereas those present on the outer surface of the cotyledons are commonly circular (fig. 3).

From the cotyledons the infection is spread to the first pair of true leaves and thence to other leaves. The occurrence of characteristic spots (fig. 4) which have manifestly arisen from infection from the cotyledons has often been noted on plants grown either in the field or in the greenhouse. These spots are first evident as small, water-soaked areas. The invaded tissues change in color to yellow, then to brown, becoming at maturity a very dark shade of brown or even purplish black. There is a retention of the translucent character, and individual lesions remain small and angular. The presence of a large number of lesions in close proximity may result in their confluence. Large, irregular dead areas may thus be formed, which may fall away, leaving perforations or notches. An indefinitely limited zone of yellowish tissue surrounds each lesion and may involve all of the intervening tissue (fig. 5). At this stage, dependent upon the severity of the infection, more or less defoliation results.

One of the distinctive features of the disease is the occurrence of a bacterial exudate on the lower leaf surface of the lesion. Under humid conditions, or if the leaves have been wet with rain or dew, turbid white droplets collect on the lesions. Otherwise, this exudate may appear as a hard, glistening film, which quickly redissolves when moisture is supplied.

THE ORGANISM

Isolation

A large number of isolations have been made from freshly invaded areas and mature lesions on leaves, from the exudate, from leaves which had overwintered in the field, and from lesions on cotyledons. Little difficulty was encountered in isolating the specific organism from any of these sources, especially after acquaintance with its appearance in culture. The organism was obtained often with an admixture of other species, but with the pathogenic form predominating, by one of the following methods:

1. The invaded areas, whose surface had been washed and disinfected in 95 per cent alcohol or 1:1000 mercuric bichlorid, were cut into pieces and laid, with aseptic precautions, upon the surface of nutrient agar plates. Abundant bacterial growth spread from the edges of these pieces of tissue. In a few cases the parasite could be transferred in pure culture

directly from these planted plates but most commonly the resultant growth was a mixture of species which were then separated by dilution cultures.

2. Small pieces cut from the lesions both directly and after surface disinfection were immersed in bouillon and after the bacteria had migrated from the tissues and diffused throughout the bouillon, poured plates were made. When proper dilutions were made isolated colonies of the parasitic form could thus be obtained in these poured plates.

3. A copious whitish exudate appeared on the lower leaf surface of areas which had been washed and disinfected. Some of this exudate was transferred by means of a sterile needle to tubes of sterile water, making a bacterial suspension from which successful platings could readily be obtained.

4. A lesion, with no previous attempt at surface disinfection, was thoroughly macerated in a drop of sterile water on a microscopic slide. A platinum loopful of this macerated material was transferred to one edge of a hardened agar plate and spread with the loop over the surface of the agar. Colonies of the causal organism, well isolated from other species, developed on these plates and were transferred directly to slant agar tubes. This is essentially the same method of isolation as is used in isolating *B. typhosus*. It is superior in the writer's hands to the other methods employed since it has yielded uniformly successful results.

Morphology

Vegetative cells. The primary cause of soybean bacterial blight is a white, rod-shaped organism with rounded ends. When taken from lesions it is found to occur singly or in pairs. In nutrient broth, however, it may form short chains of four or five elements. The individual rods measure 1.2 to 2.6 by 0.8 to 0.9 μ.

Endospores. Endospores appear not to be formed, even in old agar cultures which were stained with Zeihl-Neelson carbol fuchsin.

Flagella. When the organism from lesions, from beef broth, and from agar is examined for motility, it is seen to possess the power of rather active locomotion. This movement is made possible by the presence of polar flagella, 3 to 5 μ in length, as has been demonstrated in four strains of the organism. Use has been made of Morrey's (6) modification of Loeffler's method on 18 to 24-hour agar cultures. Usually there is a single flagellum, although individuals with two, three, or four flagella have been observed (fig. 1b).

Capsules. A well-defined capsule is readily demonstrable by Welch's method (fig. 1c), whether the parasite is taken directly from invaded tissues or from culture on solid media.

Zoogloeae. Zoogloeae have not been seen in cultures on bouillon or milk. A thin pellicle appears at the surface of liquid media, but it never becomes zoogloeae-like.

Involution forms. No involution forms have been observed.

Staining reactions. The organism stains readily with carbol fuchsin (fig. 1a), methylene blue, and gentian violet, in both aqueous and alcoholic solutions. It is not acid-fast nor retentive of Gram's stain.

Cultural characters. In the cultural studies use has been made mostly of peptone-beef agar and bouillon to which either dextrose, lactose, saccharose, or glycerin had been added. The reaction, as indicated by phenolphthalein, following boiling of the solution tested, was neutral. The several carbon compounds were made up as 20 per cent solutions, sterilized, and only added with aseptic precautions to cooled agar or bouillon in the proportion of 5 cc. to 95 cc. of media. It was thought by this means to prevent the conversion of the complex carbohydrates through heating.

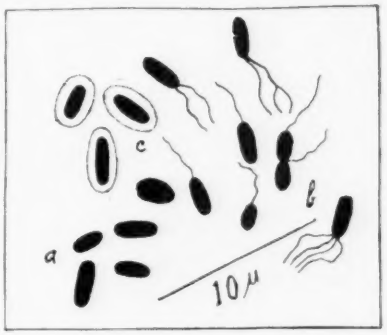

FIG. 1. *a*, *Bact. sojae* stained with carbol fuchsin; *b*, drawn from a preparation stained with Morrey's modification of Loeffler's method; *c*, the capsulate condition of *Bact. sojae* shown with Welch's method.

Nutrient agar. In agar poured plates incubated at 20 to 25°C. the colonies may appear within twenty-four hours or be first visible in forty-eight hours. At the end of four to five days the colonies are 2 to 3 mm. in diameter, circular, smooth, glistening, and possess an entire margin. They are white, raised, and soft, but not viscid in consistency (fig. 2). Buried colonies are lenticular.

On agar slants there is an abundant filiform growth in forty-eight hours, which widens at the base of the slant. The streak is opaque, raised, and glistening, with a smooth or slightly contoured topography. No odor is produced and there seems to be no change in the color of the medium beneath the bacterial growth.

In stab cultures growth occurs only at the surface. After a month's time the surface growth had covered the greater portion of the tube.

Gelatin stab. The character of growth on gelatin stabs at 18°C. is confined to the surface. Within a month the surface will have been covered, but there is no evidence of liquefaction.

Potato plugs. Abundant growth occurs on potato cylinders, more spreading and flat than on agar, and grayish-white in color. The lower portion of the streak near the surface of the water is more glistening and

more spreading than the upper part. No pronounced odor is developed and the substratum is not discolored. No change in medium is shown with iodine at the end of four weeks. A slight diastatic action in starch agar plates is evident, however, within two weeks.

Bouillon. Peptonized beef-extract bouillon becomes slightly clouded within forty-eight hours and there is little or no increase in cloudiness subsequently. A delicate pellicle is formed at the surface. No sediment is formed and no perceptible odor develops.

FIG. 2. POURED PLATE OF BACT. SOJAE ON NUTRIENT AGAR

The glistening surface with its lighter spots shows the reflection of windows

Litmus milk. The litmus milk was made violaceous in color and subjected to sterilization in an Arnold sterilizer four times at intervals of twelve hours. A week after inoculation, the blue color is intensified and approximates caeruleus. After two weeks a white precipitate is apparent, which increases as growth proceeds. Within a month, the color becomes less intense as a slight reduction of the litmus takes place.

FIG. 3. COTYLEDONS OF SOYBEAN SHOWING THE LESIONS PRODUCED BY BACT. SOJAE

FIG. 4. FIRST TRUE LEAVES OF SOYBEAN, SHOWING CHARACTERISTIC LESIONS
Photograph taken with ray filter

Physical and biochemical characters

Litmus agar. The carbon compounds, glycerin, saccharose, dextrose, and lactose from stock 20 per cent solutions, were added to cooled plain

FIG. 5. LESIONS ON SOYBEAN LEAVES

Considerable chlorosis and fusion of lesions is apparent. Use was made of ray filter. One-half natural size.

litmus agar tubes to make 1 per cent solutions of the respective sugars. Stab cultures on these media gave no evidence of acid nor gas formation after thirty days' incubation at 20°C.

Fermentation tubes. In peptone broth to which the several carbon compounds had been added no gas was formed in the closed arm of any of the fermentation tubes. There is furthermore no appreciable change in acidity. The media in the open arm becomes moderately turbid, with the most copious growth in solutions containing lactose. No growth takes place in the closed arm, indicating that the organism is strictly aerobic.

Nitrates. Tubes containing peptonized beef broth, to which 1 per cent potassium nitrate had been added, were used. At the end of five days, both control tubes and cultures were tested with starch-iodide solution. No blue color resulted indicating that there was no formation of nitrites.

Indol. Ten-day-old cultures in Dunham's solution were tested by using 0.01 per cent solution of potassium nitrite and a few drops of concentrated sulphuric acid. No pink color developed even on gently warming the tubes.

Thermal death point. The thermal death point of this organism as determined in the usual manner was found to be 52° C.

Virulence. The parasite can withstand considerable exposure, since it has been possible to isolate it from leaves which had overwintered in the field. No tests have been conducted to determine its sensitiveness in culture to desiccation, sunlight, and cold.

Group number. The group number, according to the descriptive chart of the Society of American Bacteriologists, is 212.3332023.

COMPARISON OF THE SOYBEAN BLIGHT ORGANISM WITH OTHER BACTERIAL SPECIES ON LEGUMINOUS PLANTS

Several species of bacteria parasitic on leguminous plants have been described, from all of which the form on soybeans appear to differ. It is, therefore, given a new specific name, *Bacterium sojae*, from the host upon which it is parasitic. The specific differences of the several species are summarized in table 1.

Résumé of salient characters

On the basis of the foregoing studies the parasite is manifestly distinct from species which are known to occur on leguminous hosts, and it may be briefly characterized as follows:

Bacterium sojae n. sp.[2] Rods cylindrical, rounded at ends, singly or in pairs; elements 1.2 to 2.6 by 0.8 to 0.9 μ, motile by one to several unipolar

[2] *Bacterium sojae* sp. nov., Baculis cylindraeis, apicibus rotundatis, solitariis aut geminis, $1.2 - 2.6 \times 0.8 - 0.9\ \mu$, flagello uno polare aut pluribus flagellis moti-

TABLE 1

Comparison of bacteria parasitic on Leguminosae

ORGANISM	HOSTS	AVERAGE SIZE	GROUP NUMBER
Bacterium sojae n.sp.	Soja max	2.3 x 0.8 μ	Bact. 212.3332023
Bacillus lathyrimamns and Taubenhaus	Lathyrus spp. Trifolium spp. Soja spp.	1.4 x 0.75 μ	B. 211.2222522
Pseudomonas pisi Sackett	Pisum sativum Pisum sativum var. arvense	2.2 x 0.68 μ	Ps. 211.2322033
Pseudomonas medicaginis Sackett	Medicago sativa	2.1 x 0.7 μ	Ps. 212.3332133
Pseudomonas phaseoli E. F. S.	Phaseolus vulgaris	1.5 x 0.4 μ	Ps. 211.3322513 (?)
Bacillus leguminiperdus von Oven	Pisum sativum Phaseolus vulgaris Lupinus sp.	2.1 x 0.8 μ	B. 111. . . . . (?)
Bacterium solanacearum E. F. S.	Arachis hypogoea Stizolobium deeringianum Phaseolus vulgaris	1.5 x 0.5 μ	Bact. 21½.3333823

flagella, capsulate, no endospores, strictly aerobic. Gram-negative, not acid-fast.

Superficial colonies on beef-peptone agar, circular, raised, smooth, shining, white, with an entire margin.

Gelatin not liquefied; nitrates not reduced; neither acid nor gas produced in cultures with various sugars. Group number Bact. 212.3332023.

Pathogenic on leaves of *Soja max*, forming angular brown lesions.

Type specimens have been deposited in the herbarium of the Office of Mycological Collections, Bureau of Plant Industry, Washington, D. C.

libus; capsulatis; sporis non visis; aerobicis, methodo Grami non colorantis, cum acidibus decoloratis.

Colonis superficialibus in agar-agar orbicularibus, convexis, levibus, nitentibus, albidis; gelatinis non liquefacientibus. Nitrum non reducentibus. In mediis saccharatis neque gas neque acidum evolvatis.

Laesionibus foliorum Sojae macis primum translucentibus deinde brunneolis angularisque.

INOCULATION EXPERIMENTS

The disease has been reproduced on soybeans with the characteristic symptoms, by artificial inoculation with pure cultures, in several series of experiments during 1918 and 1919. The original organism has been recovered in pure culture from five of these series and this reisolated organism has been used in four series of successful inoculations. Typical lesions followed the application of the organism to uninjured leaves. The inoculum consisted of a watery suspension of the organism and was applied by means of an atomizer.

The pure cultures used in the artificial inoculations were obtained from three sources, i.e., newly formed leaf lesions, lesions on the cotyledons, and from leaves which remained out of doors from the autumn of 1918 until March 10, 1919. Furthermore, a watery suspension has been made from the bacterial exudate taken directly from the lesions and this crude inoculum sprayed on healthy soybeans. Old leaves collected in the field during March were macerated with a mortar and pestle, covered with water for several hours and then the supernatant portion used as a crude inoculum by sprinkling it upon normal plants. Infections similar to the natural ones developed in all cases, both from pure cultures and from crude inocula. The plants used for inoculation were grown out of doors and in beds and flats in the greenhouse.

The following details will clarify the procedure employed in the inoculation experiments. A subculture was made on bouillon from a pure culture of the parasite. The cultures when twenty-four to forty-eight hours old were employed as the inoculum, after having been diluted with several volumes of water, application being made by means of an atomizer. The plants were first drenched by means of a watering pot and the inoculum was sprayed upon these wet leaves. Inoculations were made late in the afternoon and the plants were then covered with bell jars until the following morning. An incubation period of four to seven days followed. Mature lesions require two or three weeks longer for their development.

Several other leguminous plants, including cowpea, *Vigna sinensis*, velvet beans, *Stizolobium deeringianum*, bur clover, *Medicago denticulata*, and white clover, *Trifolium repens*, have been employed with negative results in artificial tests.

HISTOLOGY OF DISEASED TISSUES

For the histological studies upon the pathology of bacterial blight, lesions in various stages of development, both from natural and artificial infection, were fixed in 95 per cent alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin or preferably alcoholic methylene blue.

Entrance of the parasite is very evidently gained through the stomates. In the young infections invasion was confined to the intercellular spaces, where the organism developed so abundantly as to completely occupy the spaces and to force out the air, thus accounting for the translucency of lesions. In mature lesions the intracellar spaces are rather closely packed with bacteria. Whether the collapse of the walls and the general invasion of older lesions is due to *Bact. sojae* is not known, since a yellow, one-flagellate organism is constantly associated with the parasite in old lesions.

VARIETAL SUSCEPTIBILITY

Opportunity has been afforded, through the courtesy of the Department of Agronomy of the North Carolina Experiment Station, during the seasons of 1916 and 1917, to make observations[3] on all of the more common varieties of soybeans, including Mammoth Yellow, Haberlandt, Black Eyebrow, Tar Heel Black, Brown, Peking, Tokio, Virginia, Barchet, Chiquita, Guelph, and about 40 numbers from the Office of Seed and Plant Introduction. These varieties are grown in adjacent rows, giving ample opportunity for the spread of infection. Where evidence of difference in relative disease resistance did appear it is not believed to be due to innate qualities, although the observations are not sufficiently extended to justify passing final judgment.

OVERWINTERING AND DISSEMINATION

No observations have been made on the extent of injury from bacteria blight to the seed, but it is believed to be of no economic importance It is believed, however, that the seed are primarily responsible for the overwintering of the disease and for its introduction into new localities. Seed infection does not appear to occur until after the pods have matured and opened since lesions upon the pods have never been observed. The wetting and drying of seed in the field would at least afford ample opportunity for the organism to be washed from the leaves and to gain entrance to the seed or adhere to its surface. As has already been pointed out, lesions may occur upon the cotyledons and may be the source of infection for the primary leaves. From these the disease is gradually spread to other leaves. At least a score of field observations throughout three growing seasons add indirect evidence bearing on the persistence of the organism in the seed. Fields in which soybeans are grown for the first time are commonly more or less affected with bacterial blight. In several instances

[3] From the notes of Dr. R. O. Cromwell, formerly Assistant Plant Pathologist, North Carolina Agricultural Experiment Station.

these fields were miles from the nearest soybean field and were planted with seed from fields in which the disease is known to have been present during the previous year. Furthermore, plants coming from seed which has lain in infested fields during winter are early affected with blight. Since fallen soybean leaves decompose rather quickly, they are not believed to be responsible for infection at this time. The organism can hibernate, however, in leaves, as has been pointed out, in case the piling of the leaves prevents their decomposition.

Water is beyond doubt the chief agent for local distribution of the parasite. Beating rains could readily scatter the bacterial exudate from the surface of invaded tissues to adjacent leaves. A series of observations in 1918, in a field in which soybeans had been planted in drills eight inches apart, showed convincingly the influence of showers upon the spread of the disease. In 1919, a field which had never previously been seeded to soybeans was found, on June 12, to contain an occasional plant whose primary leaves were badly diseased. A rainy period of several days' duration followed, and on July first, when next this field was visited, nearly every plant in the field bore numerous affected leaves. Attempts to associate the spread of soybean bacterial blight with leaf-hoppers, insects commonly present on soybeans, have given negative results.

SUMMARY

Bacterial blight of soybeans is a disease whose presence appears to have been first recorded in Nebraska in 1905. It has subsequently been reported from Connecticut and Wisconsin, and the present investigation concerns its occurrence within North Carolina.

The lesions on the leaves begin as small, angular, water-soaked areas, which enlarge and change in color, becoming at length dark brown to purplish-black. The surrounding tissues are more or less chlorotic. Lesions may also be present on the cotyledons.

Under humid conditions a bacterial exudate may collect in whitish droplets on the lower leaf surface of lesions on soybean. This exudate on drying forms a film which covers the invaded area.

Infection appears to spread from the cotyledons to the true leaves and from these to other leaves.

The parasite has, on numerous occasions, been isolated from the invaded tissues and from the bacterial ooze. It is rod-shaped, flagellate, white in culture, is found to have the group number Bact. 212.3332023, and is herein described as *Bacterium sojae* n. sp.

When watery suspensions of the organism are applied to uninjured leaves infection will be evident within four to seven days.

Infection takes place through stomatal invasion, whence the organism passes into the intercellular spaces and probably becomes intracellular.

No evidences of differences in susceptibility of varieties of soybeans have been observed.

Infected seed are believed to be the chief means by which the disease is carried over winter and by which it is introduced into new localities. Leaves which remain out of doors over winter have been found to harbor this parasite. The disease is spread in the field by splashing rains.

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SOME INSECT RELATIONS OF BACILLUS TRACHEIPHILUS ERW. SM.

FREDERICK V. RAND AND LILLIAN C. CASH

WITH ONE FIGURE IN THE TEXT

The studies on insect transmission of bacterial wilt of cucurbits by Rand[1] (1915) and by Rand and Enlows[2,3] (1916, 1920), after a temporary cessation due to the war, have again been resumed, and it has seemed advisable to set forth some of the results obtained during the past season.

BEETLE DISSECTIONS AND INOCULATIONS

Previous tests had given infection[1] (1920) from the viscera, as well as somewhat less frequently from the mouth parts of a small percentage of striped and 12-spotted cucumber beetles (*Diabrotica vittata*, and *D. duodecempuncta*) fed directly upon wilted plants, when these beetle parts were directly inoculated into needle-pricked cucumber leaves. It was thought desirable to make similar tests throughout the summer using striped cucumber beetles taken from the fields at large. Collections began with the first spring appearance of the beetles in the vicinity of Washington, D. C. In no case were these insects brought into contact with the wilt organism (*Bacillus tracheiphilus*) subsequently to their collection in the fields. In cases of inoculation with the viscera alone the beetles were shaken three to five minutes in a 1–1000 solution of mercuric chloride in 75 per cent alcohol and then washed several times in tap water before dissection. Where the mouth parts also were used for inoculation they were first removed with a cold sterile scalpel, and the viscera then taken out under conditions precluding external contamination. Inoculations were then made by macerating the part to be tested in a drop of water and pricking it into a young cucumber leaf. In no case were the plants placed in damp chambers but usually the inoculations were made under

[1] Rand, Frederick V. Dissemination of bacterial wilt of cucurbits: Jour. Agr. Research **5**: 257–260, 1 pl. Nov. 8, 1915.

[2] Rand, Frederick V., and Enlows, Ella M. A. Transmission and control of bacterial wilt of cucurbits. Jour. Agr. Research **6**: 417–434, 3 fig., 2 pl. June 12, 1916.

[3] Rand, Frederick V., and Enlows, Ella M. A. Further studies of bacterial wilt of cucurbits. U. S. Dept. Agr. Bul. 828. 1920.

shade. In all cases of positive results the perfectly definite signs of the disease were further checked up by tracing the origin of wilt to the inoculation points and by a microscopical examination of the cut stems showing the vessels to be occluded with bacteria. In the first two cases *Bacillus tracheiphilus* was plated out and successfully inoculated into cucumber plants. All inoculations up to May 28 were made upon potted cucumber plants in the greenhouse at Washington, D. C., and subsequent inoculations were made at Tuxedo, Md., inside of large field cages covered with wire netting 18 meshes to the inch. The details of this series of dissection inoculations follows:

May 19: 165 male and 90 female beetles were collected from the earliest cucurbit (cymbling or scallop squash) field in the vicinity of Tuxedo, Md.; dissected and inoculated the following day. Wilt resulted from the viscera of one male and one female beetle, and *Bacillus tracheiphilus* was isolated from each of these plants and successfully inoculated again into cucumber plants. These beetles were collected fifteen to eighteen days before wilt appeared in this field or in the vicinity of Washington.

May 22: 197 males and 99 females from the same cymbling field; dissected and inoculated the following day. Wilt resulted from the viscera of one male.

May 24: 96 males and 55 females from Giesboro Point, D. C.; dissected and inoculated three days later. Results negative.

May 27: 25 males and 24 females from the same locality; dissected and inoculated the following day. One case of typical wilt started from the visceral inoculation of one male beetle but the plant was accidentally destroyed before final notes were taken.

Collections of May 28 to June 6 from cucumber and cymbling fields in the vicinity of Tuxedo, Md., and Giesboro Point, D. C., aggregating 297 males and 192 females; dissected partly on the date of collection, partly on the day following and in a few cases on the second day following; all gave negative results.

June 11: 22 males and 6 females; dissection and inoculation at once from mouth parts and viscera separately. Wilt resulted in inoculations from the mouth parts of one male and one female respectively, but in no other cases of mouth parts or viscera. These beetles were collected from two cymbling fields where an occasional natural case of wilt had developed subsequently to June 6, and about half of the beetles were taken directly from wilting plants.

June 16: 60 males and 23 females collected on healthy young cucumber plants at Greenport, Long Island, N. Y., in a field where an occasional wilting plant was to be found. These beetles were fed for five days on young bean plants (non-susceptible to wilt) before dissection and inoculation at Tuxedo, Md. The viscera of two males and one female gave typical wilt infection.

June: 18: 32 males and 7 females collected on cymbling in the vicinity of West Plains, Long Island, N. Y.; dissected and inoculated three days later at Tuxedo, Md. Results negative. No wilt was present in the field where these beetles were collected.

June 18: 70 males and 14 females from young cucumbers near Calverton, Long Island, N. Y.; dissected and inoculated 3 days later at Tuxedo, Md. Wilt resulted from the viscera of one female. No wilt was found in this cucumber field nor in the vicinity of Calverton. In fact, most of the cucurbits in this locality were just breaking through the ground.

June 21: 63 males and 38 females from the cucumber variety plats, Tuxedo, Md.; dissected and inoculated at once from mouth parts and viscera separately. Infection followed from the viscera of one male, but none from the mouth parts of any.

August 6: 50 beetles, in part recently pupated adults from the experimental plats, Tuxedo, Md.; dissected and inoculated on the same date. Wilt resulted from the viscera of one mature female.

August 18: 48 beetles in part recently pupated adults from the same field; dissected and inoculated on the same date. Results were negative.

Some of these positive results were obtained from the first beetles collected (May 19) in the earliest cucurbit plantings in one locality, whereas careful observations in this locality revealed no cases of wilt until eighteen days later (June 6), these few cases having developed in the experimental plats between the observations of June 3 and June 6. Including all cases where the sex of the beetles was recorded (up to August collections) an aggregate of 1027 males and 548 females was used. Of all positive results from visceral inoculations with these beetles 6 were from male and 3 from female individuals. Since approximately twice as many males as females were used the ratio between male and female carriers was therefore one to one.

One of the visceral infections followed from inoculation on the date of collection; four from inoculation one day after collection, one from inoculation three days, and three from inoculation five days after collection. In the latter case the beetles were fed during the interim upon young bean plants, in all other cases upon young, healthy cucumber seedlings free from beetle injuries. The greater portion of the inoculations were made one day after collection of the beetles, and with the exception of an occasional 12-spotted beetle all were striped cucumber beetles.

Out of the two sets of inoculations with mouth parts of beetles taken in part from wilting plants in the field, infection resulted from one male and one female out of an aggregate of 85 males and 44 females.

SUPPLEMENTARY EXPERIMENTS

As a check upon these dissection and inoculation tests a portion of each collection of beetles (several hundred in all) made between May 19 and 29 were enclosed with young cucumber plants in a new field cage about 40 feet square, covered with new 18-mesh wire netting and provided with a solid board base extending 18 inches into a clay subsoil. In this cage on June 11 several cases of typical wilt were found starting from beetle injuries on the leaves. On July 3 the plants in this cage were carefully gone over and 19 cases of typical wilt found—all having apparently started from beetle injuries and all unquestionably from the beetles inserted. Pure cultures of *Bacillus tracheiphilus* were obtained from several of these

plants and subsequent inoculations with these cultures were successful. It should be recalled that all of these beetles were collected and imprisoned before there was any wilt in the surrounding fields.

As a corollary to this experiment about two dozen small cone cages of 18-mesh wire netting were placed over as many young cucumber hills (June 4), the striped cucumber beetles present and gnawing the leaves first being driven away. One case of wilt, the first of the season in the vicinity of Tuxedo, Md., was observed under one of these cones on June 6, the first case in the field. On June 11 five other plants under five other cones were found wilting. All these cases had started from injuries made by cucumber beetles before the application of the cones on June 4, and no beetles had been found on any of these plants since that date. Wilt first appeared in the cucumber variety field outside of the cages on June 6 when about a dozen cases were found, all likewise having started from beetle injuries.

In all cases of natural infection seen during this series of studies (1914–1920) in which observations were made early enough in the course of the disease to determine the point of origin, the wilt clearly has always started from injuries identical with gnawings known to have been made by cucumber beetles. Experiments already reported, loc. cit. 2, 3 (1916, 1920), have shown that stomatal infections do not take place. In addition to these results, recent experiments seem to indicate that infection does not take place unless the bacteria are brought into contact with injured portions of the vascular system. In several series of experiments with young cucumber plants the epidermal and subepidermal cells were scratched with a sterile needle without going deep enough into the tissue to reach the vascular bundles. The surface of these scratched stems were smeared with virulent wilt bacteria. In no case did wilt follow although 100 per cent infection resulted when these same cultures were smeared over the deeply pricked stems or leaves of similar plants.

The results of these tests, as of the early spring dissection and inoculation experiments, indicate that a small percentage only of striped cucumber beetles harbor the wilt organism when or soon after they enter the cucurbit fields in the spring, and that the bacteria thus carried gain entrance only through fresh injuries reaching to the vascular tissues.

INTESTINAL ISOLATIONS

The dissection and inoculation tests of this and preceding seasons have demonstrated the presence of *Bacillus tracheiphilus* in the intestines of occasional striped and 12-spotted cucumber beetles. It only remained to actually culture out the organism. On November 8, 1919, twelve

striped cucumber beetles previously fed on wilting cucumber plants for two weeks were held in a clean glass jar without food for four hours. These beetles were then placed in a sterile test tube and shaken for a moment in 95 per cent alcohol and then for two minutes in mercuric chloride water (1–1000). The beetles were then washed four times in sterile tap water, placed in separate sterile Petri dishes and the viscera removed by cutting the tip of the abdomen with a sterile scalpel and squeezing out the contents onto the sterile glass by an oblique backward movement of the scalpel. The viscera from each beetle were macerated in a test tube of sterile beef bouillon, let stand for half an hour and then dilutions made in further tubes of bouillon, whereupon Petri dish cultures were made in plus 15 beef agar. In this manner *Bacillus tracheiphilus* was obtained in pure culture from the viscera of three (Nos. G, I, K) out of the twelve beetles tested. These cultures were checked up by successful inoculations into young cucumber plants.

The abdominal remains of each of these twelve beetles were macerated in a drop of water and used at once to inoculate the pricked leaves of twelve young cucumber plants, as a check on the isolation test. Typical wilt resulted from one (No. K) of these direct inoculations, and this was one of the three beetles giving the wilt organism from the visceral isolations. The juice of this wilted plant was used to directly inoculate a healthy cucumber plant which later developed the disease.

FECAL INOCULATIONS

Field observations in the early morning had many times shown what appeared to be the feces of cucumber beetles mixed in the dew over portions of cucumber leaves showing beetle injuries. Subsequently some of these leaves were found with bacterial wilt starting from the beetle injuries. Furthermore, preliminary field experiments in which different sets of caged cucumber plants were artificially inoculated by needle pricks from pure culture (1) after sundown and (2) on the following morning when it was hot and sunny, had given a higher percentage of infection from the evening inoculations. In one test all of the six plants inoculated at nightfall developed wilt, while all of the six plants inoculated the following morning remained free from the disease. These facts suggested the possibility of fecal infection in the dew over fresh abrasions on the leaves.

Accordingly a series of experiments was carried out to clear up this point. On November 8, 1919, 18 striped cucumber beetles, previously fed for three weeks on wilting cucumber plants, were placed in a clean, dry glass vial and the feces collected after two hours. This fecal matter was then macerated in a few drops of water and inoculated by needle pricks

into the leaf of a young healthy cucumber plant in the greenhouse. The results of this experiment were negative. This test was repeated, collecting the feces over night, and with a like negative result.

As a result of these two experiments it was thought possible that if the feces were not allowed to dry, and if air conditions comparable to the dew point in the field were given, a different result might follow. Accordingly on December 25, 16 striped cucumber beetles, previously fed for seventeen days on wilting cucumber plants, were caged in small vials, 4 to a vial, directly over young cucumber leaves in such a way that the feces would drop through to the leaves without allowing the beetles themselves to come into contact with the leaves (fig. 1). The cucumber leaf was supported upon a heavy cork and covered with another piece of cork $\frac{1}{8}$ inch thick. A section of this thin cork under the bottomless vial was cut out and the lower end of the vial separated from this opening through the cork by a small piece of galvanized iron wire netting, 18 meshes to the inch. The leaf was moistened, pricked with a sterile needle, and the beetles were then introduced through the upper opening of the vial, which was closed with a cork stopper and the whole apparatus held in a damp chamber over night. The presence of the cucumber leaf and the access to air below, kept the beetles most of the time near this end of the vial so that the feces readily fell through the netting and the following morning were observed on the leaf below. The beetles themselves, however, could in no way reach the leaf. The apparatus was set up at night and removed the following morning, after which the plants were held in the beetle-free greenhouse for observation. On December 6 one of the four plants was starting to wilt from the inoculated portion and three days later the whole plant had succumbed. Microscopical examination of the cut stem showed the presence of bacteria in the vessels and direct inoculation of a healthy cucumber plant with the juice from this stem induced the disease.

These same beetles were then caged for three days more on a wilting cucumber plant and the experiment repeated in an identical manner, and with the same results. One plant out of the four wilted and *Bacillus tracheiphilus* was isolated from it and subsequently checked up by successful inoculation into a healthy cucumber plant.

As a further control on this series of experiments the same set of beetles was caged over night with a wilted plant and then (December 29) removed and each of the sixteen beetles at once dissected. Inoculations were made to separate plants with the mouth parts and viscera and all held over night in a damp chamber. Four cases of wilt resulted from inoculations with the mouth parts of the sixteen beetles and five cases from those inoculated with the viscera. Two of the mouth part infections were from beetles showing no visceral infection.

FIG. 1. APPARATUS USED FOR OBTAINING FECAL INFECTIONS WITH BACILLUS TRACHEIPHILUS WITHOUT CONTACT BETWEEN INSECTS AND PLANT
Photograph by J. F. Brewer.

In a subsidiary test (December 6) where seven beetles, previously fed for eighteen days on wilting cucumber plants, were taken directly for inoculation, two cases of wilt resulted from the visceral inoculations but none from the mouth parts. These plants were not held over night in a damp chamber.

SUMMARY

It is clear that a small percentage of striped cucumber beetles harbor the wilt organism internally when they enter the cucurbit fields in the spring and there appears to be no other source for their infection than the cucurbit crop of the preceding autumn. Infections may take place from the mouth parts, at least for a limited time after feeding upon wilted plants, and infections are shown to occur when the feces of some beetles, but not all, come into contact with fresh injuries to the leaves. Infections take place only through injuries involving the vascular system. *Bacillus tracheiphilus* has been isolated directly from the viscera of wilt-fed striped cucumber beetles. Beetles of the genus Diabrotica (proved for two species) are the only carriers of the disease so far known in nature.

LABORATORY OF PLANT PATHOLOGY
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

NOTES ON SOME DISEASES OF ASPEN

CARL HARTLEY AND GLENN G. HAHN

WITH THREE FIGURES IN THE TEXT

The quaking aspen, *Populus tremuloides* Michx., probably the most widely distributed American forest tree, is in places unusually subject to disease. In the Pike's Peak region in east-central Colorado diseases are so destructive as to suggest that they may be quite as largely responsible there for the short life of aspen stands as the shading by the more tolerant conifers, to which aspen replacement in the East is commonly ascribed. The damage done by disease in the aspen in this district is not equalled in any other native American tree of which the writers have knowledge in any part of the country. Death of isolated trees as well as of those in groups regularly occurs much before maturity. Observations in the Wasatch Mountains of Utah, where aspen seems to be a less temporary forest type, indicate that diseases are decidedly less destructive there. The most prominent of the diseases noted are described in the following:

LEAF TROUBLES

The most prevalent leaf disease in the Pike's Peak region is that caused by *Sclerotium bifrons*. The disease is characterized by the death of the entire leaf. Half of the foliage over considerable forest areas is sometimes killed. The greatest prevalence is near streams. The dead leaves are typically persistent until autumn. In August one to five, or more, definitely outlined areas in each leaf become thicker and darker than the rest of the leaf, and usually drop out before the leaf falls, resulting in a shot-hole effect. The sclerotia vary from brown to black, with nearly white interior tissue. They are circular, ellipsoid, or sometimes irregularly lobed, with diameters usually between 2 and 8 mm., 3.5 mm. being a very common diameter. Attempts were made to develop fruits from these sclerotia. Fresh sclerotia were washed in mercuric chloride solution and planted in six different kinds of nutrient agar (prune, rice-stem, potato, cornmeal, beef and saccharose, and cornmeal and saccharose). The cultures either remained sterile, or yielded only *Cytospora* sp., which fruits commonly on dead aspen bark in the Pike's Peak region. Sclerotia placed on the ground in loose cloth sacks near the point of collection (9000 feet

elevation, Pike National Forest) and at Great Falls, Virginia, in the autumn, showed no change by the following spring. Possibly cultures from these over-wintered sclerotia would have been more successful.

The fungus has been found abundantly by Dr. G. G. Hedgcock and the writers on the Pike, Colorado, Rio Grande, San Isabel, San Juan, Uncompaghre, and Montezuma National Forests, Colorado, and by Dr. Perley Spaulding practically throughout New England and the Adirondack region. In Utah it has been observed, but less commonly. Specimens have been secured from the Hayden National Forest, Wyoming, collected

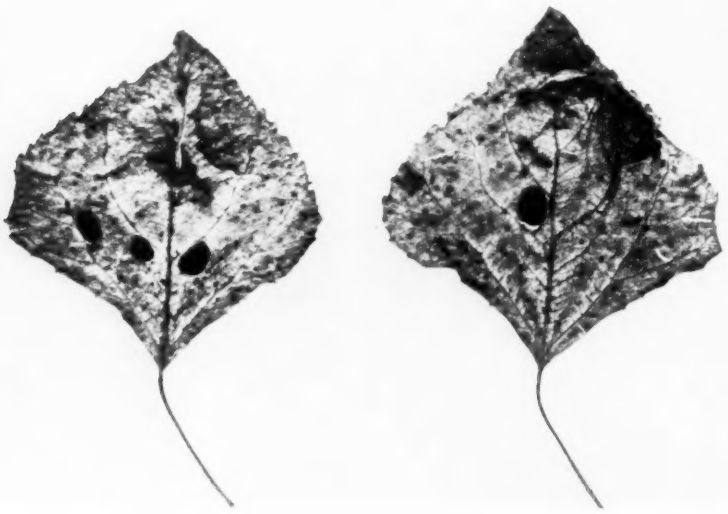

FIG. 1. LEAVES OF LOMBARDY POPLAR KILLED BY SCLEROTIUM BIFRONS

The sclerotium in the right-hand leaf has commenced the process of separation from the leaf which ultimately gives a shot-hole effect.

by Mr. A. M. Cook. In the Adirondacks, Dr. Spaulding's notes state that in mixed stands of *P. tremuloides* and *P. grandidentata* only the former is attacked. The fungus has also been reported from Wisconsin (2). Specimens have been distributed as N. A. F. 2554 (from Ontario) and Fungi Columbiana 155 (Ottawa) and 2276 (Colorado). Specimens are now in the collections of the office of Forest Pathology, collected by Dr. G. G. Hedgcock, Doctor Spaulding, Mr. G. L. Barrus, and the writers, from *P. tremuloides* in Colorado (fifteen collections), New York (three), and Vermont (two). The only collection known to the writers on hosts other than *P. tremuloides* is from Lombardy poplar (*P. nigra italica* Du

Roi), received from Miss Ethel Paine, Heath, Massachusetts (fig. 1), (Specimen F. P. 29306).

In the spring or early summer both the youngest leaves and the tender twig tips are sometimes killed in such a way as to suggest frost injury but at times when there has been little frost While the leaves do not persist and bear sclerotia, there is reason to think that this phenomenon is also due to the Sclerotium.

Cultures made from an angular leaf spot rather prevalent on the Pike National Forest yielded, in additon to hyphomycetes, nothing but *Cytospora* sp.

Leaf rust, *Melampsora albertensis* Arth. (Specimens F. P. 32698), is also quite common in the Pike's Peak region, fruiting conspicuously on the lower surfaces of the leaves, but does apparently little damage, not causing the premature defoliation often observed in young *Populus deltoides* affected with *Melampsora medusae* Thüm., or the blighting of leaves and lateral twigs by *Marsonia populi* (Lib.) Sacc. (5). This blight has been observed by Dr. G. G. Hedgcock to be frequent and injurious upon the Holy Cross National Forest, Colorado, and Targhee National Forest, Idaho.

TWIG TROUBLES

A disease locally prevalent in the Pike Forest, which has been observed in late summer during several different seasons, is a twig blight. The persistent blackened leaves give the trees much the same appearance as pear trees affected by fire blight. Insect tunnels were found in some of the affected twigs.

A trouble which may or may not have been connected with the death of twig terminals was a killing of short lateral twigs and isolated leaves with the same characteristic blackening and persistence. Examination of solitary dead leaves showed in part of the cases an insect larva tunnelling in the twig directly under the point of attachment of the petiole.

TRUNK TROUBLES

The heart rot due to *Fomes igniarius*, which is widely distributed on aspen (7), is common in parts of Colorado, entering relatively small stems and, after establishment in the trunk, spreading outward through both heart and sap wood until the cambium is reached and killed. Ordinarily however only the heart wood and older changing sap wood are affected.

The most serious damage to the aspen in the Pike's Peak region is from bark lesions. Three types are frequent: (1) Elongated dead areas most often formed on the south or west sides of trunks, presumably due to

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FIGS. 2 AND 3. POPULUS TREMULOIDES AFFECTED WITH TYPE II CANKER, BROKEN OVER AT THE REGION OF THE LESION
Photographed by Dr. Perley Spaulding, Saranac Inn, N. Y. 1909

winter sunscald. These sometimes serve as starting points for lesions such as described under III, but do not directly cause death. They have not been commonly observed. II. Determinate cankers which may appear on any side of the tree and are not believed to be connected with sun or winter injury, though such a possibility is not excluded. In size this type of canker is usually in the earlier stages small, and may become exceedingly large, lengths of 12 to 15 feet having been observed, at times practically involving the larger part of the tree In appearance these suggest cankers caused by *Bacillus amylovorus* (Burrill) De Toni. Observation indicates that some of these cankers continue to spread, successive zones of wound callus being killed. In certain cases the parasite seemed to be able to extend the lesion the second year only at the upper margin. Lesions on the trunk often originate at branch stubs. Branches as well as main trunks may be affected. The type II canker is the most serious in the Pike's Peak region as well as in the East throughout the New England and Adirondack regions. In the latter region this canker renders the aspen worthless before it reaches available size. Here the canker, according to the observations of Dr. Spaulding, causes the aspen to be readily susceptible to mechanical injury, trees frequently breaking over at the region of the lesion (figs. 2 and 3). III. Lesions on less vigorous trees, which spread more rapidly and are not often determinate or regularly zonate. These frequently cause death by girdling. All three of these types of stem lesions are common in slow-growing stands on unfavorable sites. As these sites are in general dry, it seems probable that it is the lack of vigor of the host more than favorable conditions for parasite development which is responsible for the local variations observed in prevalance of cankers. A *Cytospora* sp. with reddish or orange-colored spore horns and closely resembling *Cytospora chrysosperma* Pers. fruits abundantly on cankers of types I and III, and very promptly on aspen killed back by any agency. On cankers of type II it seems less common than on I and III. *Dothiorella populnea* Thüm. (Specimen F. P. 32699) is frequently found mixed with the Cytospora, the pycnidia crowded on a stroma, erumpent, and fully exposed when the epidermis is entirely loosened and falls away. This fungus is of interest for it is listed by Stevens (8) as being perhaps parasitic on Populus. *Valsa sordida*[1] has also been collected on the cankers.

An interesting phenomenon possibly related to a bark disease of established trees is the bark blackening of cuttings. While *Populus alba* (4) and *P. tremula* (1) cannot be propaged from cuttings, Hedrick (6) states that cuttage is a practicable method for propagating *P. tremuloides* In an effort to test the value of aspen as a nurse tree, Mr. C. G. Bates, of the Forest Service, in 1909 set out at Halsey, Nebraska, 2600 hardwood aspen

[1] Determination by Mrs. H. E. Watkins.

cuttings, part from South Dakota, part from an isolated clump of native aspen near Halsey. Both the cuttings in the nursery and those set out directly in the sandhills callused well, and at the end of a few weeks 74 per cent had started leaves. Before roots started, the bark of all became blackened in definite patches which started at the callused surfaces or at buds, spreading till the entire cutting was involved. The planting was a total loss. Cuttings taken from the Pike Forest and planted in sand in a greenhouse at Washington, D. C., behaved in the same way, though part of them had been washed in mercuric chloride solution and the ends trimmed back with a sterile knife. In this case the cuttings which had started leaf growth blackened much earlier than those which had not. Two of these cuttings commenced to blacken at points remote from buds or visible wounds, an occurrence rarely observed. The cuttings planted at Washington were set in rows, and the living and dead mapped at intervals. No evidence was secured of the spread of the disease from foci. Inner bark cut from recently blackened areas both on diseased cuttings and in cankers found on living trees was planted in nutrient media. Beef agar, cornmeal agar, prune agar, aspen agar, and beef gelatin were employed. No organism appeared consistently and many of the plantings yielded no organism whatever. Rhizoctania hyphae were common on the surface of the diseased cuttings, but inoculation at wounds with artificial cultures of Rhizoctonia, Cytospora, and Macrosporium, the two latter taken from a canker on native aspen at Halsey, failed to give results. Pieces of recently blackened inner bark were fastened in slits in healthy bark without pathogenic results.

The rapid blackening of bark is a common phenomenon in aspen killed from any cause, and therefore is not a diagnostic character for the work of any particular disease. It is entirely possible that no parasitic action was involved in this failure of the aspen cuttings. It does not seem likely that the Cytospora so commonly found was the cause of the death of the cuttings. Another possibility which seems to the writers worthy of consideration is that much of the bark killing, both on cuttings and on trunks and possibly twigs of older trees, may be due to a bacterial organism like the *Micrococcus populi* of Delacroix (3), which he was able to cultivate only in unheated poplar bark trituration. It seems especially likely that the cankers of type II may have been caused by an organism of this type.

SUMMARY

Observations have shown quaking aspen in certain areas to be unusually subject to disease; trunk cankers of unknown origin seem to be especially important factors in shortening the life of the trees. *Fomes igniarius* is

also an important factor in causing premature death of aspen in the Pike's Peak region.

Interesting but less important diseases are (1) a twig blight suggesting in appearance the fire blight of pear; (2) a leaf disease due to *Sclerotium bifrons*, E. & E., distributed from the Rocky Mountains to New England and also attacking Lombardy poplar but not *Populus grandidentata*; and (3) a rapidly spreading bark trouble which kills cuttings.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
UNITED STATES BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

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IRIS LEAF SPOT CAUSED BY DIDYMELLINA IRIDIS

W. B. TISDALE[1]

WITH SIX FIGURES IN THE TEXT

INTRODUCTION

The most widespread disease of the German group of Iris in this country is the leaf spot caused by *Didymellina iridis* (Desm.) v. H. It is rivaled in seriousness only by the bacterial soft rot of the rhizomes. Because of the increasing prevalence of the disease in the vicinity of Madison, Wisconsin, and a lack of knowledge of the life history of the causal organism and of control measures, it has seemed a problem worthy of investigation. Accordingly, the writer undertook a study of the disease in October, 1916, at the University of Wisconsin. Since that time the life cycle of the fungus has been followed, the ascigerous stage found, and successful control measures worked out. In connection with this work various details relative to the morphology and the physiology of the organism were determined, which deserve mention although not a part of the main undertaking.

THE DISEASE

History and geographical distribution

The fungus causing Iris leaf spot has been known to mycologists in foreign countries for years, but it has attracted little attention from phytopathologists until more recently. Cooke (5) seems to have been first to consider the disease of any serious import. Later (6) the same author reported the disease as being very troublesome in several parts of England and Ritzema Bos (14) reported investigations upon a disease of Narcissus which he attributed to the Iris organism. The disease occurs in practically all of the European countries (6, 11), Canada (9), and the Cape of Good Hope, but no note was made of its importance in these regions.

[1] The writer wishes to express to Dr. A. G. Johnson, at whose suggestion this investigation was undertaken, his sincere appreciation of the many valuable suggestions and helpful criticisms given during the progress of the work. He is further indebted to Dr. J. J. Davis and to Dr. C. L. Shear for their assistance in identifying the ascigerous stage of the organism, and to Dr. L. R. Jones for his assistance in the final preparation of the manuscript.

During the two years in which the problem has been under consideration, a partial survey has been made of the United States. Answers to questionnaires sent to plant pathologists of the agricultural experiment stations indicate that outside of Wisconsin the disease is prevalent only in California, Kansas, New York and Ohio. However, judging from the wide range of climatic conditions which obtain among these states, it is quite certain that the disease may develop in practically all regions where susceptible species of Iris are grown.

Hosts

At Madison, Wisconsin, natural infection has appeared on only the broad-leaved or German Iris. The species of this group which have been found affected in this vicinity are[2] *Iris germanica*, *I. florentina* var. *albicans*, *I. variegata* var. *honorabilis*, and one unidentified species. Reports from European countries show that the same fungus crosses over to other species and genera of hosts and Cooke (5) reported it on Lychnis, a member of a widely separated family. Ritzema Bos (14) attributed a serious disease of several varieties of Narcissus to the same fungus. In 1888 Ellis collected, at Newfield, New Jersey, a fungus which he identified as *Heterosporium gracile* Sacc. on *Hemerocallis fulva*. The same organism has been reported by Lindau (11) as occurring on Freesia, Antholyza, Gladiolus, and Gemmingia. Since the only specimens readily available for examination were those of broad-leaved Iris, no comparative study of the organisms from the various hosts was made. Cross inoculation experiments to *Hemerocallis* sp., *Narcissus* Emperor, and Pheasant (Hort. varieties) with the organism from Iris were unsuccessful in every case. This would seem to indicate specialized races or even different species of the fungus on the various host genera.

Signs of the disease

The disease has been observed on the leaves only, with infection most abundant on the apical half. It is first evident as minute brown spots surrounded by a watersoaked margin which later turns yellow (fig. 1). The spots occur on either surface of the leaf, but are not visible on the opposite surface until one or two days later. In the spring the spots enlarge slowly, requiring about thirty days to become ¼ inch in diameter. Later in the season, however, they enlarge much more rapidly and produce conidia after about two weeks. The size and the number of the spots

[2] Bailey's Standard Cyclopedia of Horticulture has been followed in the above classification and nomenclature.

vary with the species of host; they are about ¼ to ½ inch in diameter on *I. germanica* and smaller and more numerous on the other species. The older spots are oval or elliptical in shape, with the longer axis parallel to the veins. They may in some instances be so numerous as to kill the entire leaf early in the summer, but, as a rule, only a few of the leaves die before the blossoming season. It is not uncommon to find a striping of the leaves of *I. germanica*. This is due to dying of the tissue between the spot and the apex of the leaf. The older spots have a grayish center which is dotted with tufts of black conidiophores (fig. 2). Surrounding this center is a reddish brown border which is a deposit of some substance on the cell walls. The nature of this substance was not determined. The descriptions given by Cooke, Güssow, and Ramsbottom correspond very favorably with the above. It is, therefore, presumed that they were dealing with the same hosts.

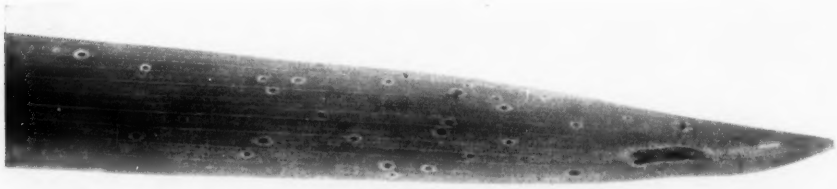


FIG. 1. IRIS LEAF SPOT TEN DAYS AFTER ARTIFICIAL INOCULATION

According to Ritzema Bos (14), the disease of Narcissus, for which he holds the same fungus responsible, attacks the plants in quite a different manner. He states that the first evidence of the disease is a yellowing of the margins of the leaf, which soon involves the whole leaf, thereby causing the sudden death of the affected parts. After the leaf dies its upper surface becomes covered with a blackish fungus growth. The leaves of Iris are very similar morphologically to those of Narcissus, and it seems unlikely that the same organism would produce only a spot on the former and completely destroy the leaves of the latter.

Economic importance

Didymellina iridis reaches its most destructive stage at such a period in the growth of the plant that it is difficult to estimate the amount of damage incurred in any one year. Although the disease appears soon after the young leaves emerge in the spring, it does not become very notice-

able until about the blossoming season, which in Wisconsin is the latter part of June. After this time the disease develops very rapidly and kills many of the leaves prematurely. This premature death of the foliage reduces the amount of food stored up in the rhizomes, and if the process continues from year to year the death of the plants will ultimately follow. The disease has not been followed closely enough and for a sufficient

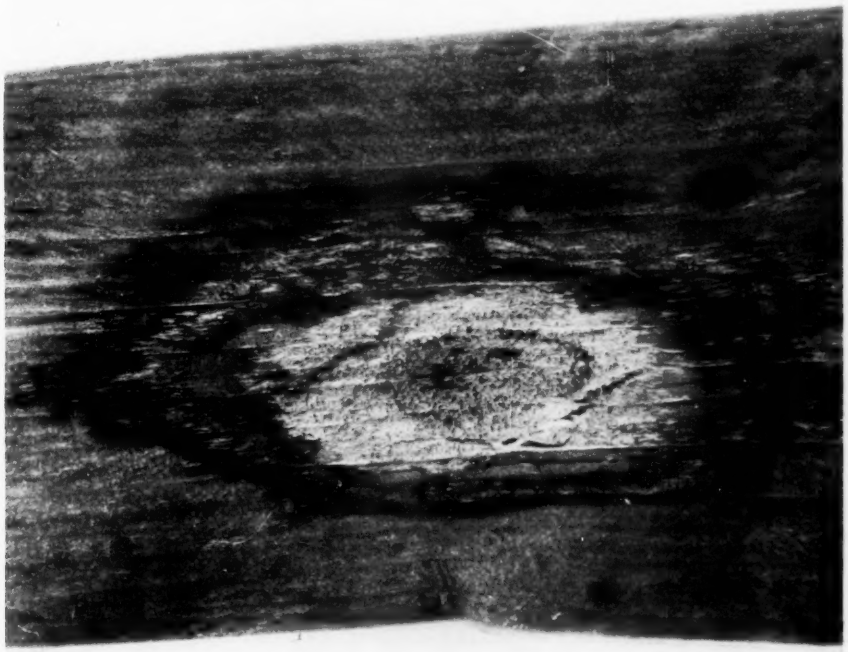


FIG. 2. OLD SPOT ON IRIS LEAF, SHOWING TUFTS OF CONIDIOPHORES
Natural infection. × 5

length of time to determine to what extent the vigor of the plants is thus reduced from year to year.

Cooke says (6): The leaf spot is one of the most troublesome and persistent diseases of Iris in England, the plants being killed in one or more years. Güssow (9), on the other hand, considers the disease of minor importance in Canada.

Pathological histology

In all cases examined the germ tubes were found to enter the tissue through the stomata (fig. 3). Ramsbottom (13) states that he found penetration taking place both through the stomata and directly through the epidermis. Ritzema Bos (14) states that the fungus attacking Narcissus penetrates the tissue by both methods. Inasmuch as not a single case of direct penetration of the cuticle was found, it seems reasonable to believe that this mode of attack is the exception rather than the rule.

Upon entering the substomatal chamber the germ tube sends out branches which wedge between the cells, forcing them apart. Later the hyphae become intra-cellular, the cells collapse and the affected area dries out. The contents of the affected cells first turn yellowish and then

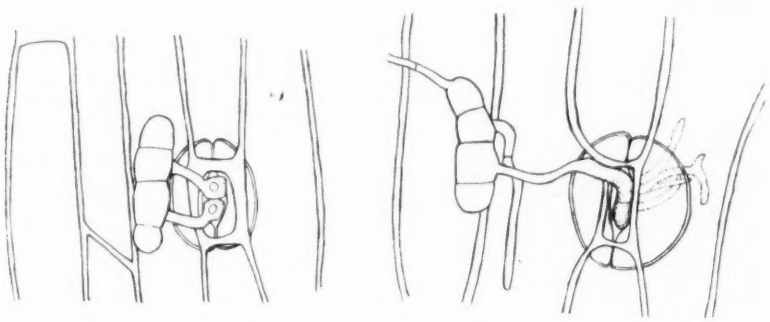


FIG. 3. DIFFERENT STAGES OF STOMATAL PENETRATION BY CONIDIAL STAGE OF DIDYMELLINA IRIDIS

Showing germination characteristics of conidia

gradually disappear. A reddish brown material accumulates on the cell walls in the margin of the affected zone, giving the characteristic brown margins. Small masses of hyphae develop in the substomatal chambers and give rise to tufts of conidiophores, which in crowding through the stomata tear the epidermis loose from the underlying tissue, thus giving the chracteristic gray color to the center of the spot. The hyphae seem to be restricted to the spot while the leaf is yet green, but after the leaf dies they spread throughout the tissue.

THE CAUSAL ORGANISM

Taxonomy

Conidial stage. Early records of the fungus on Iris date back to 1881, when Saccardo (15) described the conidial stage of the organism and referred it to *Heterosporium echinulatum* (B. & Br.) Cooke. This specific

name had previously been given by Berkeley (2) to the fungus attacking carnations. Later Saccardo (16) referred the fungus on Iris to *Heterosporium gracile* Wallr. He had, however, already referred the *Helminthosporium gracile* Wallr. to *Brachysporium* (16, p. 430). This, according to current description, is a very different fungus from the one under consideration here. On this account later authors as Cavara (3), Schroeter (17), Oudemans (12), Ramsbottom (13) and others have attributed the name *Heterosporium gracile* to Saccardo.

Ascigerous stage. During the course of these studies an ascomycete was found on the dead, infected leaves of *I. variegata* var. *honorabilis* and *I. florentina* var. *albicans*. Pure cultures obtained from single ascospores of this fungus have produced typical conidia, and the positive results of inoculation experiments with such conidia seem to be sufficient proof that the ascomycete under consideration is the ascigerous stage of *Heterosporium gracile*.

There seems to be no record in literature of any previous connection of *H. gracile* Sacc. with an ascigerous stage. Also, judging from description only, the ascigerous stage under consideration seems to differ from any species of sphaeriaceous forms attacking Iris.

Specimens were, therefore, sent to Dr. C. L. Shear at Washington, D. C., for identification. Upon comparison he found them to be identical with specimens distributed by Desmazieres in his Pl. Crypt. France, No. 94 (1853) on leaves and pods of *Iris pseudacorus* collected at Hermanville, France, by Roberge, and described as *Dothidia iridis* Desm. in Ann. Sci. Nat. Bot., Ser. 3, VIII Bd., p. 76, 1847. He also compared them with specimens collected by Roberge at Caen, France, and now in the herbarium of Michener.

Von Höhnel (10) in "Mycologische Fragmente," CCVII in Annales Mycologici, vol. XVI, nos. 1 and 2, 64–66, 1918, enters into a full discussion of the morphology and taxonomy of *Dothidia iridis* Desm. This author says (p. 66) that *Dothidia iridis* Desm. is a Didymella Sacc. (1878) without paraphyses and therefore belongs to a new genus which he calls Didymellina. He also claims that many species of Mycosphaerella belong to this genus. Accordingly, he applies the binomial *Didymellina iridis* (Desm.) v. H. and lists the following as synonyms:

Type species: *Dothidia iridis* Desm. 1847.

Synonyms: *Sacidium desmazierii* Montagne 1857
Sphaerella iridis Auersw. 1869
Metasphaeria iridis (Desm.) Sacc. 1883
Mycosphaerella iridis (Auersw.) Schroeter 1894
Sphaerulina iridis (Desm.) Berlese 1894
Sphaerella desmazierii (Mont.) Sacc. 1895
Sphaerella desmazierii (Roberge) v. H. 1910

Some confusion exists among mycologists as to the taxonomic position of some of the above forms, or else von Höhnel included forms which should not be considered as synonymous with the specimen originally described by Desmazieres. A comparison of specimens of *Sphaerella iridis* Auersw. and *Mycosphaerella iridis* (Auersw.) Schroet. in the herbarium of the Department of Botany of the University of Wisconsin with the fungus described in this article reveals a great difference in size of perithecia and of spores. The perithecia of these specimens measure only about 120 to 160 microns in length and the spores 4 to 5 by 12 to 17 microns. Two of the herbarium specimens which were on green leaves collected in June and September showed no evidences of a conidial stage. The spots had no definite reddish brown border and were more irregular in shape than those upon the material collected at Madison. As stated elsewhere, perithecia have been collected at Madison on only the dead leaves of Iris and only during the spring months.

Morphology

The fungus, *Didymellina iridis* (Desm.) v. H., has two stages in its life cycle, namely, (1) the conidial stage parasitic on the leaves of Iris, and (2) an ascigerous stage which develops in the spring on the dead leaves infected during the previous season. The perithecia were found to be sterile two years out of the three during which the fungus was under observation and so appear to play a relatively unimportant part in the life cycle.

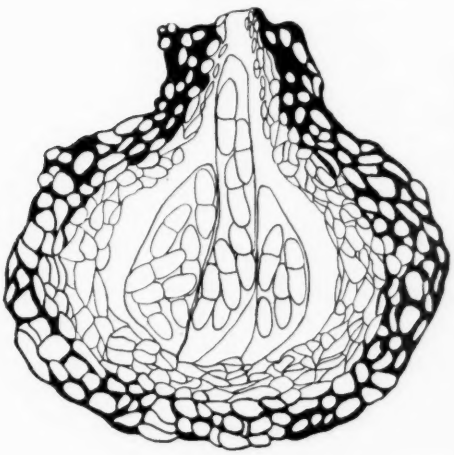
Conidial stage. Three or four weeks after primary infection occurs, tufts of conidiophores emerge through the stomata in the diseased spots and bear conidia. The conidiophores are olivaceous and more or less zigzag in shape at the upper end. The dimensions vary considerably with environmental conditions. The ones borne in the spots during the summer average about 11 by 70 microns, while those developing in the spring on the apices of sterile perithecia average about 12 by 150 microns.

The conidia are echinulate, concolorous with the conidiophores, cylindrical with rounded ends, two to three septate, slightly constricted at the cross walls, and range from 13.7 to 18.7 by 40 to 60 microns, averaging 16.8 by 50.8 microns. The range in size is quite variable in any one spot or in pure culture, and while the range is a little greater than that given by Saccardo and Lindau, it does not seem sufficient for specific separation.

Ascigerous stage. Perithecia scattered, smooth, globose, dark brown to black, 130 to 220 by 188 to 246 microns, averaging about 160 by 200 microns; subepidermal, with a papilliform ostiole which may bear a tuft of conidiophores under favorable weather conditions; walls pseudoparenchymatous, two to four cells thick (fig. 4).

Asci obclavate to oblong, hyaline, thin walled, rounded and obtuse above, tapering below into a short pedicel, or sessile, fasicled; 36 to 54 by 72 to 117 microns, averaging 49.9 by 97.2 microns; from 6 to 25 in a perithecium, without paraphyses (fig. 5).

Ascospores oval-elliptical, one septate, distinctly constricted, contents light greenish-yellow, wall slightly tinted; 10.5 to 16.2 by 30.6 to 54 microns, averaging 14.4 by 44 microns, slightly unequally two-celled; usually eight spores in an ascus.



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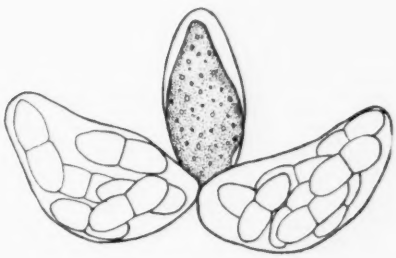

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FIG. 4. DIDYMELLINA IRIDIS

Camera lucida drawing of a median section through a perithecium

FIG. 5. ASCI OF DIDYMELLINA IRIDIS

Showing different stages of maturity in the same perithecium

Physiology

Cultural studies. In pure culture the young hyphae are grayish-white, delicate and septate. On most media used this type of hyphae forms a dome-shaped, aërial colony within the first few days. After about five days the hyphae begin to form a thin, dark brown to black stroma on the surface of the medium from which olivacious conidiophores arise and produce conidia acrogenously. As each conidium approaches maturity it is pushed aside and the conidiophore continues to grow and produce more conidia until from two to four are produced. The conidia are very easily dislodged and are never found with more than two *in situ*.

The fungus grows well on most of the common laboratory media except beef-peptone agar. It also grows well on steamed stems of *Melilotus alba* and steamed leaves of Iris. As compared with the Fusaria and some other organisms, it does not grow very vigorously on any of the media, requiring about two weeks to cover the surface of a slanted tube of agar. It produced conidia more abundantly on potato agar, corn meal agar, and sterilized stems than on any of the other media used.

On steam sterilized stems of *Melilotus alba* and steamed Iris leaves the growth is at first similar to that on other media except that conidia are produced two or three days earlier. Perithecia begin to develop when the culture is about twelve days old, and reach their maximal size about ten days later. During the first week they contain abundant oil globules, but these finally disappear and the perithecia remain sterile. Such cultures were treated in many different ways in an attempt to stimulate the production of ascospores, but without success.

Germination studies

Conidia. Conidia taken from a pure culture germinate readily on both solid and liquid media. Germ tubes are produced from any or all of the

TABLE 1

The relation of temperature to the rate of conidia germination

TEMPERATURE	TIME REQUIRED FOR GERMINATION
deg. C.	*hours*
4	21
8	6
13	4
17	3
22	2½
26	2½
30	11
35	No germination

cells of each spore, and as many as three tubes may be produced from a single cell. The germ tubes from a single spore may develop simultaneously, or later in the middle than in the end cells. Spores germinate best and most rapidly at 20° to 26°C., as shown in table 1. At temperatures higher or lower than these germination takes place more slowly, and at 30° the tubes are densely vacuolate and grow very slowly.

The optimal temperatures (22–26°C.) commonly obtain out-of-doors during the latter part of May and June, when the disease is developing most rapidly.

Ascospores. Ascospores removed from perithecia and planted in water or on water agar germinate within about six hours. Development of the germ tube is in some cells immediately preceded by the appearance of a septum, and each of the three or four cells thus formed is capable of producing one or two germ tubes (fig. 6). Once started, the tubes grow fairly rapidly on potato agar and after five days a colony developing from a single ascospore produces conidia which are exactly similar to those of *H. gracile.* The colonies developing from single ascospores are likewise similar to those developing from conidia, when grown on the same kind of medium.

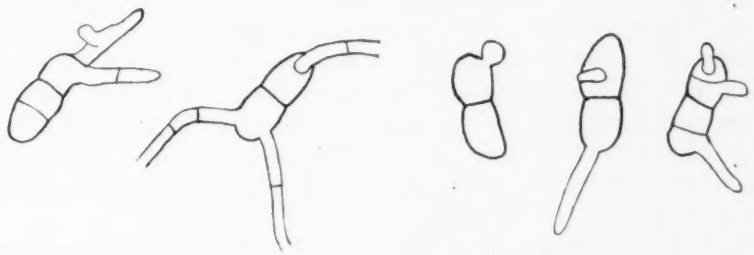

FIG. 6. GERMINATING ASCOSPORES OF DIDYMELLINA IRIDIS

Overwintering and life history of the organism

The causal organism of Iris leaf spot passes the winter in the mycelial stage in the old infected leaves, and produces conidia and perithecia early the following spring. During the course of these studies no viable conidia were found in the field from December to March. However, an examination of the dead leaf tissue revealed the presence of dark brown, thick walled hyphae and when the leaves were brought into the laboratory and kept in a moist chamber at room temperature for two or three days abundant conidiophores and conidia developed in the old spots. Conidia developed in culture at 8°C. in fifteen days, and were found outside in March, following a few days of warm weather. This seems to indicate that successive crops of conidia are produced throughout the winter in sections having a milder climate than that of Wisconsin. Since the conidia germinate within twenty-four hours at 4°C., it seems reasonable to suppose that, unless subjected to extremely low temperatures, they are short lived in nature.

Late in February and early in March small masses of dark colored hyphae appear beneath the epidermis in lesions on old leaves and later develop into perithecia. The outcome of the perithecia seems to be dependent upon weather conditions. In the spring of 1917 they matured

about April 1, but in all cases were found to be sterile. Most of these sterile perithecia bore a tuft of four to twelve conidiophores on the apex, or ostiole. This peculiar manner of fructification is probably due to the fact that the sudden change from cold to warm temperature was unfavorable for the production of asci. During the spring of 1918, which was somewhat similar to that of 1917, a few perithecia were found to contain asci and ascospores. The spring season of 1919 opened rather early and was followed by intermittent cool periods which continued well into April. Under such weather conditions one would expect the fungus to react quite differently than it did the two previous years with different weather conditions. At any rate, the old leaves which were taken from the field about March 30 bore numerous perithecia containing mature asci and ascospores. These fertile perithecia bore no conidiophores at the apex, as did the sterile ones the two previous years. Such erratic occurrence of ascospores indicates that they play a minor part in the early infection of new leaves. On account of the difficulty experienced in obtaining a number of ascospores free from segments of hyphae and conidia, no inoculations were made with them directly.

Since there is always an abundant supply of newly developed conidia by the time the young leaves appear, it is probable that they serve as the chief source of primary infection. In infected beds the new leaves come into direct contact with the old infected leaves and thus, incidentally, are sprinkled with a plentiful supply of viable conidia. Perhaps rain, too, aids in distributing the spores. The primary spots begin to appear about the middle of April and after about a month, depending upon the weather conditions, are producing conidia, which, in turn, serve to continue the infection. After the blossoming season the spots become numerous on all susceptible species and many of the leaves are thus killed down before frost.

INOCULATION EXPERIMENTS

The inoculation experiments with the Iris leaf spot organism were conducted during the spring seasons of 1917 and 1918. Practically all of the experimental work was done in the greenhouse to insure against outside infection. Rhizomes from which all of the dead infected leaves were removed were brought in from the gardens during the winter and planted in pots in the greenhouse. These plants grew vigorously and apparently normally, and no signs of disease developed on the uninoculated plants at any time. The experiments were conducted along two lines, namely: (1) inoculation of the varieties of Iris which were attacked by the fungus outside, and (2) cross inoculation to *I. siberica*, *Narcissus* varieties Emperor and Pheasant and one unidentified species of Hemero-

callis. After inoculation the plants were kept in a moist chamber for about thirty-five hours and then placed in a greenhouse room where the temperature was held fairly constant at about 22°C.

Three methods of inoculation were used, namely: (1) insertion of fragments of mycelium into the leaf tissue with a sterile needle, (2) sowing the conidia in drops of sterile water on marked areas of the leaf surface, and (3) spraying the plants with a sterile water suspension of conidia.

TABLE 2

Results of the inoculation experiments with H. gracile

KIND OF PLANT	KIND OF INOCULUM	NUMBER OF PLANTS INOCULATED	NUMBER OF PLANTS INFECTED	PERIOD OF INCUBATION	INFECTION RESULTS
				days	
I. germanica	Mycelium	3	3	2	+
I. germanica	Mycelium	4	4	2	+
I. variegata var. honorabilis	Mycelium	3	3	2	+
I. variegata var. honorabilis	Conidia	2	2	5	+
Hemerocallis sp.	Conidia	2	0		−
Hemerocallis sp.	Mycelium	2	0		−
I. siberica	Conidia	4	0		−
I. siberica	Mycelium	3	0		−
Narcissus var. emperor	Mycelium	2	0		−
Narcissus var. emperor	Conidia	4	0		−
Narcissus var. pheasant	Conidia	3	0		−
Narcissus var. pheasant	Mycelium	2	0		−
Hemerocallis sp.	Mycelium	3	0		−
I. germanica	Conidia	3	3	8	+
I. germanica	Conidia	6	6	5	+
I. florentina var. albicans	Conidia	2	2	5	+
I. florentina var. albicans	Conidia (ascospore strain)	3	3	6	+
I. germanica	Conidia (ascospore strain)	2	2	8	+

In practically every case all three methods gave positive results when susceptible species of Iris were used. Previous to inoculation with conidia, the heavy bloom was removed from some of the leaves by lightly rubbing them with a piece of clean cheesecloth. Water adhered better to the leaves treated in this manner than to untreated leaves and they always showed heavier infection. The results of the inoculation experiments are given in table 2.

The inoculation experiments show that the varieties of German Iris are very easily infected, while *I. siberica*, Narcissus, and Hemerocallis are evidently immune. Where mycelium was the source of inoculum, infection took place very rapidly with susceptible hosts and after about six days the spots were about ½ inch in diameter and were bearing conidia. Infection from conidia occurred in from five to eight days and developed very slowly at first, attaining a diameter of from 1 to 3 mm. in thirty days. The lesions developed more rapidly on plants which were placed outside of the greenhouse as soon as infection took place.

The failure of attempts to produce infection in Narcissus and Hemerocallis suggests the probability of different strains or species of the fungus. The varieties of Narcissus used in these experiments are listed by Ritzema Bos (14) as being susceptible to the disease with which he worked and which he attributed to *H. gracile*. This organism has also been reported by mycologists as occurring upon Hemerocallis both in America and in Europe. No strains of the organism were obtained from any other host than the species and varieties of German Iris herein mentioned and so far as these experiments justify conclusions, it is very likely that cross inoculation experiments with the organism from different hosts will show the existence of different strains or even of different species.

CONTROL MEASURES

The writer has been unable to find any record in literature of successful control measures for the Iris leaf spot. Güssow (9) and Cooke (6) each recommended the removal of the dead infected leaves but did not show any evidence that the method had been successfully employed. Ramsbottom (13) stated that the limeloving species of Iris were the only ones affected with the leaf spot and that they were attacked only when grown on soil deficient in this substance. He therefore planned some experiments based on this theory, but for some reason never reported upon execution of them.

After the development of the fungus had been carefully followed during the winter and spring of 1917 at Madison, Wisconsin, it was thought advisable to try two methods of control, namely: (1) removal of dead leaves prior to the development of young leaves in the spring, and (2) protection of plants with a coating of some copper fungicide during their early development.

On March 30, 1917, the dead leaves were removed from two small beds of *I. germanica* and one of *I. variegata* var. *honorabilis*. However, by this time the leaves of *I. germanica* were from 1 to 2 inches long and conidia were being produced in small numbers on the dead infected leaves. One

of the beds of *I. germanica* was sprayed with 4-4-50 Bordeaux mixture. At the same time and subsequently at intervals of two weeks until June 20, the spray mixture was applied to one small bed of each of the above named species from which the leaves had not been removed. No signs of infection developed on the plants in any of the three beds from which the leaves were removed until late in the summer, and then only a few lesions appeared. It is likely that the spores causing this infection came from nearby diseased beds. The disease developed somewhat later on the sprayed beds from which the leaves were not removed than upon the unsprayed controls, but was more abundant and widespread than upon the beds from which the leaves had been removed. This indicates that primary infection was prevented but that the plants were not continuously protected by the spray mixture. On account of the heavy bloom on the Iris leaves, it is a difficult problem to get the spray mixture to adhere uniformly. Also, as the plants approach the flowering stage it becomes very difficult to reach all parts of the leaves with the spray solution. Since the conidia are present on the dead leaves until summer, the plants would have to be kept continuously covered with the spray in order to insure protection.

Since the above difficulties had been encountered in 1917, it was decided to resort only to the removal of dead leaves as a possible means of control in 1918. Immediately after the snow had melted, two beds of *I. germanica*, the dimensions of which were about 8 by 10 feet, received this treatment. These plots were located in the horticultural gardens and far enough away from other plots to insure against infection from other sources. Previous to June 15 no signs of the disease had appeared in either of the treated plots, while in the check plots abundant infection was found by the first of May. Furthermore, Professor L. R. Jones employed in his private garden, which was planted with *I. germanica* and *I. variegata* var. *honorabilis*, the method used in these experiments, with the result that no signs of the disease had appeared in either of these beds up to June 15. Probably this method will prove effective for controlling the disease in all regions where the leaves die down during the winter. Under these climatic conditions the dead leaves are easily removed in a practical way by simply giving the plants a thorough raking or "combing" with a steel garden rake. In the warmer climates where the leaves remain green over winter, the method may have to be modified or used in combination with some spraying program.

SUMMARY

1. Leaf spot is one of the most widespread diseases of the broad-leaved or German group of Iris in the vicinity of Madison, Wisconsin.

2. The disease is present wherever the susceptible species of Iris are grown. In the United States it has been reported as of considerable importance in California, Kansas, New York, Ohio, and Wisconsin. Cooke reports it as one of the most serious and troublesome diseases of Iris in England.

3. The groups of Iris other than the broad-leaved group and also the species of Narcissus and Hemerocallis used in the experiments are evidently immune from the disease.

4. The disease is caused by a fungus the conidial stage of which has been known for some time as *Heterosporium gracile* Sacc. The ascigerous stage of the organism has been found at Madison, Wisconsin, and has been referred to *Didymellina iridis* v. H.

5. The fungus overwinters in the mycelial stage in the dead leaves. Perithecia develop early in the spring, but do not always produce asci, apparently on account of the weather conditions. Sterile perithecia bear conidia on the apex.

6. An abundant crop of conidia, always present early in the spring, serves as the chief source of primary infection.

7. The only mode of penetration found during the experimental work at Madison was through the stomata.

8. The removal of dead infected leaves before the young leaves appear in the spring promises to be a successful means of control.

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THREE WINTER WHEAT VARIETIES RESISTANT TO LEAF RUST IN KANSAS[1]

LEO E. MELCHERS AND JOHN H. PARKER

WITH THREE FIGURES IN THE TEXT

INTRODUCTION

Observations on the occurrence of the leaf rust of wheat, *Puccinia triticina* Eriks, have been made at Manhattan and in other parts of Kansas with particular reference to the comparative susceptibility of varieties during the seasons of 1914–1919, inclusive. These observations have shown that in certain localities leaf rust may be present in quantities sufficient to cause actual damage (3).

The leaf rust of wheat as it occurs in Kansas may or may not show the same biologic specialization as the leaf rust occurring in other regions. There is also, the possibility that more than one biologic form has been present in the experimental plots during the six-year period in which the data were obtained, though the results with the varieties do not definitely indicate it. Some caution, at least, is necessary in presenting and interpreting the effects produced by any cereal rust in any one locality, in view of the known existence of several distinct biologic forms of stem rust of wheat, as described by Stakman and Piemeisel (6), Melchers and Parker (5), and Levine and Stakman (2).

WINTER WHEAT VARIETIES RESISTANT TO LEAF RUST

In the investigations conducted at Manhattan, resistance to leaf rust has been observed in the same varieties which show a marked degree of resistance to one form of stem rust, namely, *Puccinia graminis tritici* (4). These varieties are Kanred, P762 (C. I. 5146) (Kansas No. 2401); P1066 (C. I. 5879) (Kansas No. 2415); and P1068 (C. I. 5880) (Kansas No. 2414). All these varieties belong to the Crimean hard winter wheat group and are bearded with glabrous, white, smooth chaff and hard, red kernels.

[1] Coöperative investigations between the Kansas Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture. Published with the approval of the Director, as a contribution of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, paper No. 30.

Kanred (1) has been distributed by the experiment station to farmers and is probably the best variety of hard winter wheat now available for Kansas conditions. The other two varieties seem fully as promising in every

TABLE 1

Data showing infection by leaf rust on three varieties of hard, red winter wheat and on adjacent rows of other winter wheats, at Manhattan, Kansas, during the six years from 1914 to 1919, inclusive

YEAR	VARIETY	AVERAGE PERCENTAGE		MAXIMUM ON ANY VARIETY
		On resistant varieties	On varieties in adjacent rows	
1914	P762 Kanred	10.0	40 and 65	
1914	P1066	7.0	70 and 50	90
1914	P1068	7.0	40 and 60	
1915	P762 Kanred	10.0	40 and 25	
1915	P1066	5.0	25 and 40	65
1915	P1068	5.0	40 and 25	
1916	P762 Kanred	15.0	25 and 30	
1916	P1066	5.0	50 and 30	70
1916	P1068	5.0	50 and 30	
1917	P762 Kanred	10.0	40	
1917	P1066	5.0	37 and 32	65
1917	P1068	3.0	32 and 25	
1918*	P762 Kanred	5.5	77 and 79	
1918*	P1066	8.5	82 and 76	95
1918*	P1068	9.0	70 and 78	
1919†	P762 Kanred	6.7	79 and 73	
1919†	P1066	6.6	66 and 66	90
1919†	P1068	5.0	71 and 66	With very heavy infection on the sheath and peduncles

	per cent
Six-year average for P762 (Kanred)	9.5
Six-year average for P1066	6.2
Six-year average for P1068	5.7

* The rust percentages for this year are averages of a larger number of rows (pure line selections) grown in the rust nursery.

† The rust percentages for this year were taken from three series of variety plots at the agronomy farm, in addition to those obtained in the botany rust nursery. All varieties, with the exception of these three, showed from a trace to very heavy infection on the sheaths and peduncles.

respect, judging from records on yield, winter survival, and other characters obtained in the agronomy nursery. They have not, however, been compared in field plots, or in the cooperative experiments with farmers in the different sections of the state.

The observations on leaf rust infection presented in this paper were made in the rust nursery maintained by the Department of Botany and Plant Pathology, in the agronomy nursery and in commercial fields. The figures in table 1 are averages from a large number of plants, taken from rows in different parts of the nursery. The estimates of the amount of infection were made in accordance with the scale for estimating rust

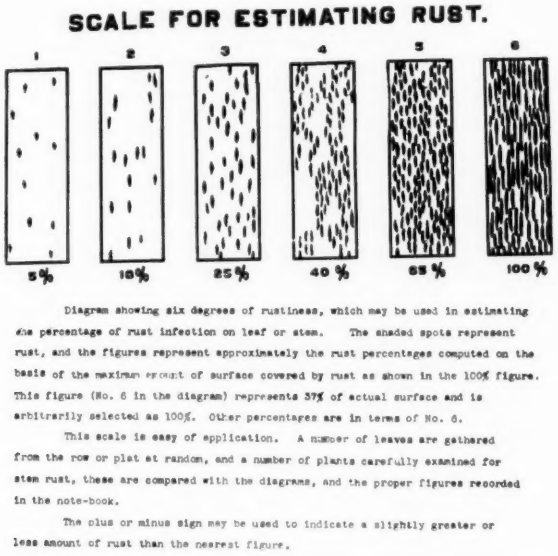
SCALE FOR ESTIMATING RUST.
1
2
3
4
5
6
5%
10%
25%
40 %
65 %
100 %
Diagram showing six degrees of rustiness, which may be used in estimating the percentage of rust infection on leaf or stem. The shaded spots represent rust, and the figures represent approximately the rust percentages computed on the basis of the maximum amount of surface covered by rust as shown in the 100% figure. This figure (No. 6 in the diagram) represents 37% of actual surface and is arbitrarily selected as 100%. Other percentages are in terms of No. 6.
This scale is easy of application. A number of leaves are gathered from the row or plat at random, and a number of plants carefully examined for stem rust, these are compared with the diagrams, and the proper figures recorded in the note-book.
The plus or minus sign may be used to indicate a slightly greater or less amount of rust than the nearest figure.

FIG. 1. SCALE USED FOR ESTIMATING RUST PERCENTAGES

which is used by the Office of Cereal Investigations of the United States Department of Agriculture and is shown in figure 1.

It will be seen that the amount of leaf rust on the three varieties described as resistant was consistently less than on other varieties grown in adjacent rows and very much less than the maximum amount recorded on the most susceptible varieties grown in the nursery. It should be added that, during each season, the maximum percentage of leaf rust recorded was not confined to one or two varieties, but that there generally were a number on which the amount of infection reached this maximum, while a still larger number bore leaf rust percentages closely approaching the maximum figure.

The six-year average infection on Kanred, P762, is 9.5 per cent; on P1066, it is 6.2 per cent; and on P1068, only 5.7 per cent. These averages are remarkably low and indicate that the three varieties possess a high degree of resistance, even when grown under optimum conditions for infection. These conditions may be considered as the final test of the resistance or susceptibility of any variety, at least as far as its immediate practical value is concerned.

Figure 2, *A* and *A'*, shows the characteristic infection of leaf rust on the leaves of a susceptible variety, P706 (Kansas No. 2382), Improved Turkey. The uredinia are numerous and entirely normal. The leaves from which the illustrations were made were typical of this variety and were collected from plants in rows adjacent to P762, P1066, and P1068. Figure 2, *B* and *B'*, shows leaves of the resistant variety Kanred, P762. There are few uredinia, but many light yellow or green flecks, indicating the presence of rust hyphae, but failure to form uredinia. Figure 3, *C* and *C'*, shows leaves of another resistant variety, namely, P1066. There are few uredinia but many sharp flecks. Figure 3, *D*, and *D'*, shows two leaves from the third resistant variety, P1068. There are few uredinia, but the flecks, characteristic of the resistant varieties, are very distinct. The discolored brown areas of leaf tissue are due to the drying of the leaf from other causes and should not be confused with the leaf rust. The specimens shown in the illustrations were collected in June, 1918, from plants grown in the rust nursery, where a uniformly heavy infection of leaf rust was present. The varieties were approaching maturity and the leaf rust infection had unquestionably attained its maximum development.

In 1916 leaf rust was very prevalent in many wheat fields in the state and the infection was particularly conspicuous because it occurred on the peduncles or necks and sheaths of the plants, as well as on the leaf blades. A field of P706, Improved Turkey, at the agronomy farm near the college, showed an actual reduction in yield which, in the opinion of members of the Department of Agronomy, resulted from the heavy attack of leaf rust. A field of P762, Kanred, grown on the same farm had only a light infection on the leaf blades, recorded as 15 per cent, and no uredinia on the necks. The yield of this variety was not appreciably affected. The resistance to both leaf and stem rust was one factor which aided the Department of Agronomy in deciding to distribute P762, Kanred, instead of P706, Improved Turkey, known to be susceptible to both rusts.

Additional evidence on the resistance of P762, Kanred, was obtained in the agronomy nursery during the summer of 1918. An experiment on the date of planting as related to the habit of growth was located near the other nursery plantings and contained the following varieties: Defiance Winter (Kansas No. 373), Currell (Kansas No. 2406), Turkey (Kansas

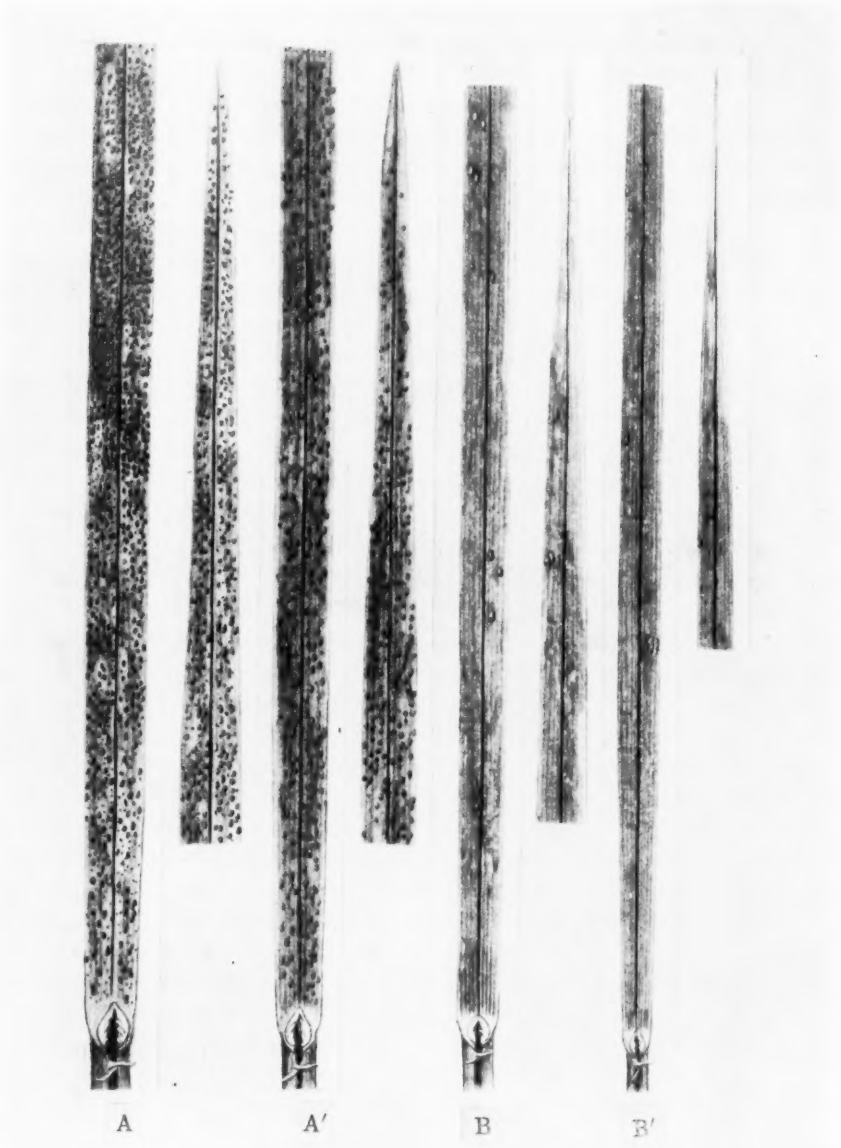

FIG. 2. *A*, The characteristic infection of leaf rust on the leaves of a susceptible variety P706 (Kansas No. 2382), Improved Turkey; *B*, the leaves of the resistant variety P762 (Kansas No. 2401), Kanred. Note, only a few uredinia, but numerous flecks, denoting resistance.

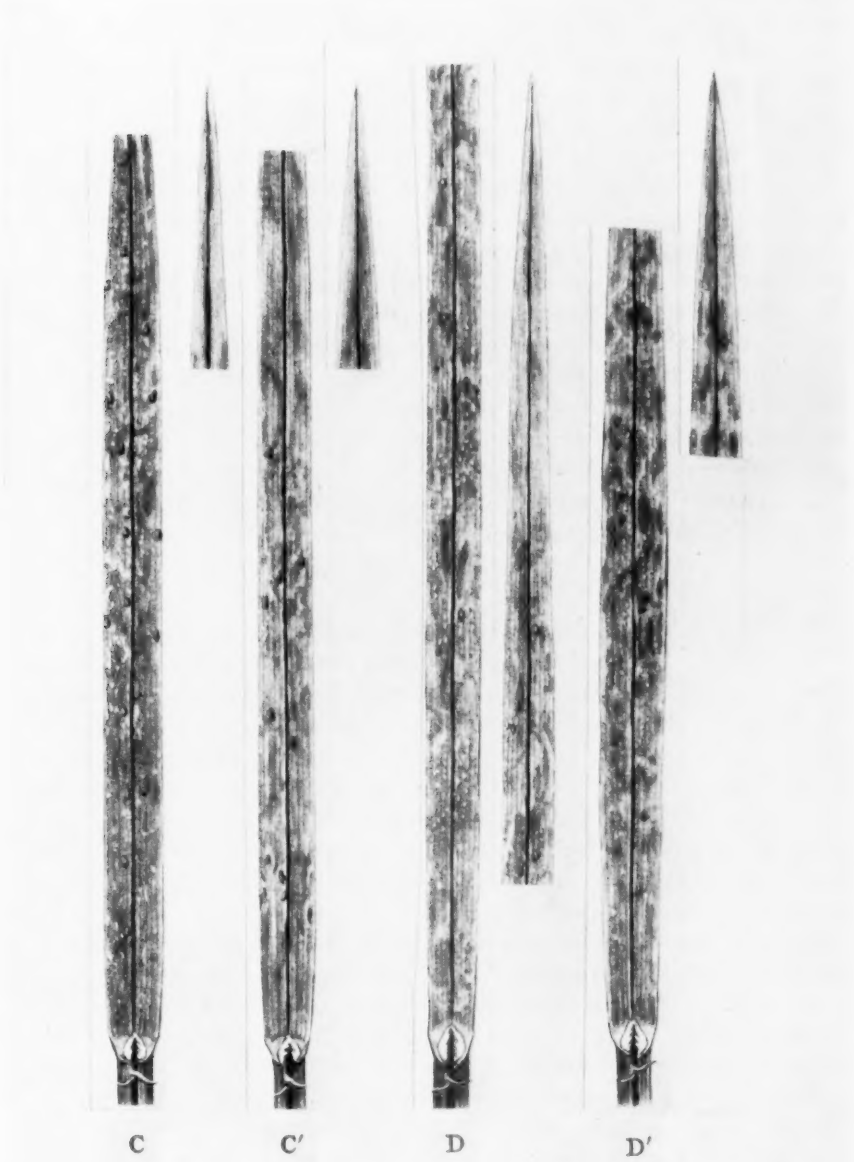

FIG. 3. *C*, leaves of the resistent variety P1066 (Kansas No. 2415), note, few uredina, but many sharp flecks; *D*, leaves of a third resistant variety, P1068 (Kansas No. 2414). Note, few uredinia, but many flecks which are characteristic of the resistant varieties. The discolored brown areas of the leaves are not due to leaf rust infection.

No. 570), Kanred (Kansas No. 2401), Fultz (Kansas No. 2156), and Nebraska No. 28 (Kansas No. 34). These were planted on the first of each month and in the order mentioned. The rows of Kanred were located between Turkey (Kansas No. 570), a hard wheat, and Fultz (Kansas No. 2156), a soft wheat. Both of the latter varieties are known to be susceptible to leaf rust. The plantings which were made on June 1 and July 1 were making vegetative growth during the ripening of the wheats in the regular nursery and after they were harvested. There was an abundance of natural infection of leaf rust in the nearby regular nursery plantings, and these were scattered and continued to spread on the green plants in the date of planting experimental plots. A heavy infection was present in these rows throughout the late summer and early fall and the amount of leaf rust on the plants of Kanred was not more than 20 per cent, while that on the adjacent rows and all the other varieties, varied from 50 to 85 per cent. There were some uredinia on the leaves of the Kanred plants, but flecks were much more numerous. These observations made on late plantings materially strengthened the evidence of the resistance of P762, Kanred, for a variety sometimes shows resistance when planted at the normal time, but fails to maintain this resistance when planted on a later date.

In addition to the observations made at Manhattan, there has been evidence from the reports of cooperating farmers that fields of Kanred grown in various parts of the state are much less affected by leaf rust than are fields of other varieties commonly grown on the same farm. These reports were definitely confirmed the past season. In the Kanred seed inspection work which was carried out in fields of Kanred wheat which were grown in Kansas the past season, a thorough examination was made for both leaf and stem rust. A most severe epidemic of leaf rust occurred in all parts of the state during the spring of 1919, which afforded the best opportunity to see what the behavior of Kanred would be in Kansas when grown on a commercial scale. Specimens were gathered from about 46 counties representing more than 500 fields. Notes were taken on the percentages of leaf rust, not only of Kanred, but of all adjoining fields of wheat of other varieties. The percentages for Kanred were strikingly uniform, ranging between 8 and 10 per cent, while the percentages of leaf rust for adjoining fields of other varieties which served as checks, varied from 20 to 95 per cent, the majority ranging close to the latter figure. The sheaths of Kanred plants were always entirely free of any leaf rust sori, while all other varieties showed from slight to heavy infection. It has not been possible to obtain as extensive records of P1066 and P1068, since these varieties have not been distributed to farmers but the indications are that they will be just as resistant as Kanred.

Experimental plantings of the three varieties made at certain stations in other states have also had a lighter infection of leaf rust than local varieties grown under the same conditions. These varieties must be grown at these and other stations for a series of years, however, before final conclusions as to their resistance in these localities can be reached.

AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

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BRIEFER ARTICLES

THE DISTRIBUTION OF BUCKEYE ROT OF TOMATOES

J. L. Weimer

The buckeye rot of tomato fruit, caused by *Phytophthora terrestria*, was first described by Sherbakoff from Florida in 1917. He had knowledge of its existence in southern Florida since 1915, and preserved specimens found in the laboratory of the Florida Agricultural Experiment Station indicated that it had been prevalent as early as 1911. This disease was reported to the Plant Disease Survey from Arkansas and Porto Rico in 1917 and from Texas and Louisiana in 1918. Bruner states that what he supposed to be *Phytophthora terrestria* was found causing a rot of green tomatoes in Cuba in 1918. So far as available records show, this disease is present only in tropical or subtropical regions. The purpose of this note is to record its occurrence in Alexandria County, Va., on the United States Department of Agriculture experimental farm at Arlington, Virginia, in 1919.

During the latter part of August several green tomatoes showing the typical brown lesions with the concentric rings, as illustrated by Sherbakoff, were found. Other specimens were obtained at different times throughout the remainder of the season not only in this but also in two other patches, each being a considerable distance from the other. In the patch where the first specimens were found considerable damage resulted to the fruit in contact with or very close to the soil. In no case, however, was the fungus found causing a disease of the stems or leaves. Isolations were made and a Phytophthora conforming to the description of *P. terrestria* obtained, and its parasitism proved by inoculations made from pure cultures into tomatoes. This organism was also compared with an authentic culture of *P. terrestria* from Florida.

Buckeye rot of tomatoes, then, is not restricted to the extreme Southern States, as previous reports on its occurrence might seem to indicate.

Bureau of Plant Industry
Washington, D. C.

VARIETAL SUSCEPTIBILITY TO FALSE BLOSSOM IN CRANBERRIES

S. B. FRACKER

On a recent examination of nursery inspection reports, the writer was impressed with the marked difference between the varieties of cranberries in the amount of "false blossom" found on the bog. The variation was so striking that an analysis of the reports for the past five years was made and it is presented herewith.

The nursery inspection office of the State Department of Agriculture of Wisconsin was requested in 1915 to provide for the inspection of cranberry vines and to grant certificates in the same way that other plants and plant products for propagation are handled. An accusation that certain growers were planning to dispose of vines in which false blossom was present was the original cause of this action, and this disease condition has been the principal cause for refusing to grant inspection certificates to cranberry growers, although occasionally the presence of insect pests has resulted in similar action.

The so-called "false blossom" condition has been described by Shear[1] and by Jones and Shear.[2] In Wisconsin it is the cause of a serious loss in production. The symptoms are the development of what appears to be a blossom, followed by a green shoot instead of a berry. The pedicel of the flower remains erect instead of bending over, as normal blossoms do, and it is said to be distinguished from lack of pollination in this manner.

No organism responsible for the trouble has been isolated, and there is little evidence to show that the condition is an infectious disease. It is fairly common in Wisconsin bogs which have been planted to berries of the so-called "Berlin" group, although varieties not belonging to this group have also developed the same condition. An idea of its prevalence may be gained from the fact that it has been necessary to refuse permits for the sale of plants from an average of 54 acres per year or about 20 per cent of the 252 acres inspected.

The acreage of the varieties inspected and the per cent of false blossom found are shown in the following table.

Two extremes of susceptibility are apparent. The native Wisconsin varieties, Bell and Cherry and Metallic Bell are so uniformly victims of this condition that the source of plants of these varieties has become greatly reduced. In fact the situation is shown too conservatively in the table, for large acreages in which false blossom was found in 1915

[1] Shear, C. L. Cranberry diseases. Bul. 110, Bur. Plant Ind., U. S. D. A. 1907.

[2] Jones, L. R., and Shear, C. L. A report upon false blossom and other cranberry maladies. Rept. 27th Anl. Mtg. Wis. Cranb. Gr. Assn. 1914, pp. 13, 14.

have been withdrawn from inspection and no plants have since been sold from these bogs. Less than half an acre of Metallic Bell a year, 6 per cent of the inspected area of this variety, has been licensed for sale. The 44 per cent of the Bell and Cherry acreage shown represents less than 10 per cent of the bogs from which Bell and Cherry might be sold were it not for false blossom.

At the other extreme are the more vigorous growers, McFarlin, Searls Jumbo, and Prolific, with practically clear records. Bennett Jumbo seems to be comparatively resistant while the acreages of Potter's Favorite, Early Ohio, and other varieties examined have been too small to show positive results.

This varietal susceptibility might be considered as due to the different cultural conditions under which the different varieties are grown, but this

Table showing false blossom acreage

VARIETIES LISTED IN ORDER OF TOTAL ACREAGE INSPECTED	AVERAGE ACREAGE INSPECTED ANNUALLY	AVERAGE ACREAGE INFECTED ANNUALLY	PER CENT DISEASED	TOTAL NUMBER OF INSPECTIONS MADE 1915–1919	NUMBER OF OWNERS OF INFECTED PLANTS
Searls Jumbo	79	0	0	18	0
Bell and Cherry	60	34.0	56	17	8
Bennett Jumbo	50	11.0	22	16	8
McFarlin	37	0.2	1	14	1
Prolific	7	0.4	6	16	1
Early Ohio	7	1.4	20	4	2
Metallic Bell	6	5.6	93	14	12
Others and mixed	6	1.6	27	16	5
Total	252	54.2	21		

appears unlikely owing to the large number of different bogs upon which each variety was observed. It is the opinion of Mr. O. G. Malde, who has made all the cranberry inspections for the department, that improper cultural conditions from which Bell and Cherry and Metallic Bell suffered years ago have resulted in deteriorated stock, but whether false blossom could be developed and inherited under such circumstances is questionable. Cultural conditions do greatly modify the severity of the attack, for the disease is much more common on wet bogs than on the drier type. The application of commercial fertilizer and sanding of the bogs have also resulted in an improvement even where false blossom was already present.

Even though the cause may be a physiological one, plants from an infected bed continue to develop false blossom after being transferred to new locations. The loss usually then becomes greater from year to year and total crop failure has sometimes resulted, followed by the abandon-

ment of the bogs. Under these circumstances the nursery inspection office must adopt the same policy in providing for cases of false blossom as if the disease were proven to be of an infectious nature.

We are recommending to cranberry growers: (1) that they choose resistant varieties of strong, vigorous growth for new plantings; (2) that they take vines for new plantings from beds without a trace of false blossom; (3) that they adopt cultural methods which will secure the largest returns in healthy bogs, for these are also the best ones for assisting affected plants to overcome the false blossom condition.

STATE DEPARTMENT OF AGRICULTURE
MADISON, WISCONSIN

OBSERVATIONS ON SEED TRANSMISSION OF THE CABBAGE BLACK ROT ORGANISM

J. C. WALKER AND W. B. TISDALE

Since its presence on cabbage seed after harvest was first demonstrated by Harding, Stewart, and Prucha,[1] the transmission of the cabbage black rot organism (*Bacterium campestre* (Pam.) Smith) in this manner has been generally assumed. These investigators further showed that when seeds were bathed in a water suspension of the organisms and allowed to dry, some of the latter were still viable and pathogenic after eleven months. Since conclusive evidence of the organism living over from one season to the next on naturally infected seed is either lacking or very meager in the literature, observations strengthening this point and also giving evidence of the efficacy of seed disinfection are offered in this note.

The disease appeared in epiphytotic form in a large number of fields in the Racine, Wisconsin, district in 1919. When first observed on August 14 in a field of Copenhagen Market variety, it was well advanced and of such long standing that many plants had been prevented from heading. Part of this same field was planted with a late variety which came from a different source and these plants were practically free from disease. Further survey of this region during the remainder of the season showed many fields with general infection, while in numerous instances another variety in the same field was entirely healthy.

Inquiry as to the source of seed in each case revealed the fact that in every instance where black rot developed in epiphytotic form the seed had been secured from the same local seedsman. This dealer, it was learned, had distributed two lots of 1918-grown seed in the spring of

[1] Harding, H. A., Stewart, M. C., and Prucha, M. J. Vitality of the cabbage black rot germ on cabbage seed. N. Y. (Geneva) Agr. Exp. Sta. Bul. 251, p. 177–194. 1904.

1919, both of which had been imported during the previous winter. These consisted of one lot (1) of Hollander variety, and one lot (2) of Copenhagen Market. Every field examined where seed from either of these two lots had been used showed more or less black rot.

A similar condition was found in the vicinity of Appleton, Wisconsin, where one dealer distributed a lot of imported seed of the Hollander variety (lot 3). In the two fields examined where seed from this lot had been used, black rot was very prevalent. Moreover in both instances plants from another lot of seed which had been used alongside the imported strain were practically free from disease except in rows immediately adjacent to the latter, where recent spread had occurred.

TABLE 1

Summary of field observations on the occurrence of black rot in the Racine, and Appleton, Wisconsin, districts in 1919

LOCALITY	FIELD NUMBER	VARIETY	SEED LOT NUMBER	DATE OF OBSERVATION	PER CENT OF PLANTS INFECTED WITH BLACK ROT	PER CENT PREVENTED FROM HEADING BY BLACK ROT
Racine	1	Copenhagen	2	August 14	95	60
	2	Copenhagen*	2	August 29	1	
	3	Hollander	1	September 1	100	94
	4	Hollander	1	September 1	59	
	5	Hollander	1	October 13	95	
	6	Hollander*	1	September 1	12	1
	7	Hollander*	1	October 14	1	0
Appleton	8	Hollander	3	October 10	74	13
	9	Hollander	3	October 10	60	23

* Seed treated before planting with 1:1000 mercuric chloride for thirty minutes.

In several instances both in the Racine and Appleton sections, plants of the infected and the uninfected strains had been grown in the same seed bed, thus confirming the assumption that the pathogen had been introduced with the seed rather than by way of the soil. A summary of the observations in various fields is given in table 1. Spread of the disease from infected portions of fields to healthy plants of different origin in adjoining areas became very apparent several weeks after transplanting. In the case of field 3, for instance, counts were made in an adjacent plot of cabbage grown from clean seed. On September 1, eight to nine weeks after transplanting, the row next to the plot of infected Hollander showed 98 per cent of the plants with marginal infections of fairly recent origin; the second row showed 67 per cent; the sixth row 66 per cent; and the

twelfth row 18 per cent. It is thus clearly evident that the black rot organism is readily disseminated in the field by natural agencies. In field 3 the disease progressed so rapidly that most of the plants were prevented from heading. In other fields where a large percentage of the plants headed, the soft rot which followed did considerable damage before harvest or caused heavy losses in transit.

It should be noted that in the three cases where the seed was treated before sowing with mercuric chloride, according to directions sent out by the Wisconsin Experiment Station, the disease was well controlled.

UNIVERSITY OF WISCONSIN
AND
BUREAU OF PLANT INDUSTRY
MADISON, WISCONSIN

RESULTS OF WHITE PINE BLISTER RUST CONTROL IN 1919

S. B. DETWILER

Blister rust has made steady progress in the generally infected white pine region of New England and New York. Extensive areas of severely infected pines exist in most of these States. In a 41-acre pine lot at Littleton, New Hampshire, containing a total of 5442 native white pines averaging 6 inches in diameter, breast height, 55.4 per cent of the trees were recently found infected with the rust. This infection is entirely due to wild Ribes (currants and gooseberries). Wild gooseberries scattered over the 41-acre plot average 30 bushes per acre, 199 feet of leaf-bearing Ribes stems per acre. Strip surveys on compass lines a rod wide, totalling $22\frac{5}{8}$ miles in length, were run out from this pine lot in five directions across the surrounding country. Out of 2856 pine trees growing on this strip, 25.4 per cent were infected.

These data indicate that one-fourth of the pines in an area approximately 9 miles long and 8 miles wide are already infected with the disease. The oldest blister rust canker found on pine in this region is estimated to have started in 1906. These figures show that pine infection is progressing more rapidly than was estimated previously.

Control of the disease through destruction of currant and gooseberry bushes has been tried experimentally since 1916, in coöperation with the New England States and New York. The results in 1919 show that such control is practical and effective in protecting a given area of pines. On four control areas from which currant and gooseberry bushes were removed in 1916 and early in 1917, careful scouting this year failed to reveal any new pine infections occurring since the Ribes were destroyed. Under competent direction, unskilled laborers can be quickly trained to find and

destroy over 95 per cent of the wild currant and gooseberry bushes the first time they go over the control area. In 1919, accurate checks were made on 1070 check plots containing over 750,000 bushes, to determine the thoroughness with which Ribes eradication was done. As part of their training, the crews made 642 checks, in which a total of 246,254 bushes, or 96.4 per cent, were uprooted in the first working, 8095 the second time over the ground, 905 the third time, 125 the fourth time, and 10 the fifth time. On 6 areas worked in previous years, totalling 1725 acres, 152,942 bushes were found in the original working and only 6474 bushes were found in checking. This is an efficiency rating of 95.9 per cent for the first working. Of the bushes found in checking, 4105 (or 2.6 per cent of the total found) were obviously missed when the areas were first worked; and 2369 (or 1.5 per cent of the total found) were sprouts and seedlings that had developed after the first working. On an acre basis, 89 Ribes bushes were destroyed in the first working, 2 bushes were missed, and 1 new plant appeared after the work was done. On 65 plots where the number of bushes was counted before and after the crews worked the areas, and where the men did not know their work was being checked, they found and destroyed 96 per cent of the bushes. Federal and state inspectors made numerous other checks which also show that the crews destroy in excess of 95 per cent of the bushes in the regular course of their work. Some of the check plots were gone over 10 to 15 times in order to be certain that no plants were missed.

It was found that on an area where only 87 per cent of the Ribes bushes were taken out in the first working, these bushes bore 96.6 per cent of the leaves. In this test, the area was gone over four times, and the leaves on 2194 bushes were counted. In the first working, 94,429 leaves were destroyed, and in the other three workings, only 3094 leaves were found. Measurements of the annual growth of 836 wild gooseberry bushes gave an average height growth of 5 inches per year for four years. Other counts showed that less than 5 per cent of the wild gooseberry bushes produce fruit, even in favorable crop years. Intensive study of Ribes seedlings on small plots demonstrate that they summer kill as well as winter kill, so that only a very small percentage live through the first year.

The cost per acre of Ribes eradication is low. The State crews eradicated 4,265,000 Ribes from 241,590 acres in 1919, at an average cost of 54 cents per acre. In 1918 the average cost was 66 cents per acre on areas totalling 135,239 acres. This reduction in cost was secured through scientific methods of locating Ribes in regions where they are not generally distributed. By this means, the average cost per acre in New Hampshire was reduced from 39 cents last year to 21 cents this year. In Rhode

Island this method reduced the cost from 27 cents per acre in 1918 to 11 cents per acre in 1919. The cost per acre in New York was much greater, due to an average of 92 wild currant and gooseberry bushes per acre, whereas in New Hampshire the average was only 10 bushes per acre. It is confidently expected that further experimental work will result in greatly reducing the cost without decreasing the efficiency of the work. In this connection, three years' experimental work in spraying Ribes with various chemicals indicates that dip oil and fuel oil, alone or in combination, are effective in destroying Ribes. Where the bushes are very numerous, the cost of eradication by chemicals is apparently much less than by hand pulling.

About $10,000 for coöperative Ribes eradication in New York in 1919 was subscribed by individuals and associations. In New Hampshire, 53 towns appropriated a total of $8514 for coöperative eradication of Ribes, and 34 individuals and firms subscribed an additional $2053. In Massachusetts, local coöperators furnished $1075. The sympathetic attitude of the public toward blister rust control is further evidenced by the fact that in New Hampshire 21,171 cultivated currant and gooseberry bushes were destroyed, and that compensation, as provided by law, was asked for by only three owners.

The necessity for developing local control of the blister rust is apparent from the rate of increase of the disease in native pine stands; and the demonstrated practicability of Ribes eradication fully justifies pine owners in undertaking this work on a large scale. Studies conducted by the Office of Forest Pathology, Bureau of Plant Industry, on the distribution of sporidia and the distance that infection has actually occurred upon pines from known infected Ribes, indicate that the Ribes-free zone should be, under average conditions, 200 to 300 yards in width. Tests of the longevity of the sporidia showed that they remained viable for a period of less than ten minutes where the humidity was 90 per cent.

Recommendations relative to control measures are summarized in the following extract from the resolutions of the Fifth International Blister Rust Conference, held at Albany, New York, on December 8–9, 1919:

> The Fifth International Blister Rust Conference urgently recommends the complete destruction of all Ribes within a distance of 200 to 300 yards of native and planted pines in regions where blister rust is present or within dangerous proximity. Owing to wide variations in the distance that spores spread from Ribes to pine, due largely to the nature of the vegetation intervening between the Ribes and pine, and to weather conditions, it is impossible to establish a fixed width of Ribes-free zone which will be equally applicable to all localities; but it is the opinion of this Conference, based upon recent investigations, that a Ribes-free zone of 200 to 300 yards in width will be sufficient to insure the commercial growing of white pine under average conditions. It is also recommended that white pine should not be planted in the locality of large plantations of Ribes, especially cultivated black currants.

Scouting was continued in the Middle Western and Southeastern States where the white pine is native and planted. The disease was found only in single plantations in Michigan and New Jersey. An area of disease approximately 150 miles long and 100 miles wide was found to exist in Wisconsin and Minnesota. At a conference held at Saint Paul, Minnesota, in October, 1919, to which representatives of the Lake States and Canada were invited, the conclusion was reached that the policy in these two states should be local control of the disease on the same basis as in New England.

A number of violations of the Federal quarantine against shipment of Ribes and white pines into the Pacific Coast and Rocky Mountain States were discovered in 1919. These states have been well scouted for four years and no blister rust has been found; but unless nursery stock of five-needled pines and of Ribes from infected regions is absolutely excluded from the far West, it is practically certain that the blister rust will eventually be introduced there. The fact that 65 species of wild Ribes are native to western North America and constitute a large proportion of the undergrowth in much of the western white pine and sugar pine forests, makes it imperative that the blister rust be kept out of this region if humanly possible to do so.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

LITERATURE ON PLANT DISEASES[1]

COMPILED BY ALICE C. ATWOOD AND FLORENCE P. SMITH, BIBLIOGRAPHICAL ASSISTANTS, BUREAU OF PLANT INDUSTRY

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